Aus der Tierklinik für Fortpflanzung
des Fachbereichs Veterinärmedizin der Freien Universität Berlin

Calving prediction and evaluation of calving ease after medical treatment in Holstein-Friesian heifers

Inaugural-Dissertation
zur Erlangung des Grades eines
Doktors der Veterinärmedizin
an der Freien Universität Berlin

vorgelegt von
Katrin Susanne Lange
Tierärztin aus Hannover

Berlin 2020
Journal-Nr.: 4171

Aus der

Tierklinik für Fortpflanzung

des Fachbereiches Veterinärmedizin

der Freien Universität Berlin

Calving prediction and evaluation of calving ease after medical treatment in Holstein-Friesian heifers

Inaugural-Dissertation

zur Erlangung des Grades eines

Doktors der Veterinärmedizin

an der

Freien Universität Berlin

vorgelegt von

Katrin Susanne Lange

Tierärztin aus Hannover

Berlin 2020

Journal-Nr.: 4171

Gedruckt mit Genehmigung des Fachbereichs Veterinärmedizin
der Freien Universität Berlin

Dekan:	Univ.-Prof. Dr. Jürgen Zentek
Erster Gutachter:	Univ.-Prof. Dr. Wolfgang Heuwieser
Zweiter Gutachter:	Univ.-Prof. Dr. Jörg Aschenbach
Dritter Gutachter:	Univ.-Prof. Dr. Rudolf Staufenbiel

Deskriptoren (nach CAB-Thesaurus): holstein-friesian, heifers, calves, calving, birth, veterinary practice, lactates, lactic acid

Tag der Promotion: 21.09.2020

Meinen Eltern

In den Wissenschaften ist viel Gewisses, sobald man sich von den Ausnahmen nicht irremachen läßt und die Probleme zu ehren weiß.

Johann Wolfgang von Goethe

TABLE OF CONTENTS

1 INTRODUCTION

Regularly calving is essential for dairy cows to continue producing milk. Possibly, calving is the most important moment in the life of a dairy cow (Villettaz Robichaud et al., 2017a). Good calving management intends so ensure a smooth calving procedure. As definitions of calving assistance scores are not consistently used on farms and in literature, frequency of calving assistance in literature is varying (Villettaz Robichaud et al., 2017a). In a study investigating three Colorado dairy operations with 7,380 calvings per year, 51.2 % of the primiparous and nearly 30 % of the multiparous dams required calving assistance (Lombard et al., 2007). In case calving assistance is required, prediction of calving is an essential prerequisite.

Several methods exist which are currently used for calving prediction. Also, technical devices are used to measure temperature (Burfeind et al., 2011), rumination activity (Pahl et al., 2014) or acceleration (Ouellet et al., 2016). Additionally, a combination of all three methods is possible (Rutten et al., 2017). As a relatively current possibility, birth monitoring systems, e.g., an intravaginal sensor (iVet©, Papenburg) are available, but there is still need for improvement (Henningsen et al., 2017). However, there is no fully satisfactory method with regard to effort, costs and ability for prediction.

Alleviating calving is especially important when problems during parturition occur. One agent that regulates myometrial contractions during parturition is denaverine hydrochloride (**DNH**) (EMA, 1997). To our knowledge, hardly any blinded, randomized trial testing DNH under farm conditions is published.

The objectives of these studies were to predict stage 2 of calving and to examine the impact of DNH on calving ease and calf vitality. Data from the first study were used for research paper 1, data obtained during the second study were basis for research paper 2 and research paper 3, respectively.

1.1 Calving prediction in heifers

The process of parturition can be divided into three stages (Noakes et al., 2001). Stage 1 is defined as the preparation stage during which the birth canal and the fetus are prepared for expulsion. The structure of the cervix alters to enable dilatation, myometrial contractions start and the fetus changes position (Noakes et al., 2001). Additionally, several behavioral changes occur. Stage 2 of calving starts with the onset of abdominal contractions and the appearance of the amniotic sac outside the vulva and ends with the delivery of the fetus

(Noakes et al., 2001; Schuenemann et al., 2011). The third stage of parturition comprises the expulsion of the fetal membranes.

At present, a wide variety of methods for calving prediction exists. Still, there is no method available that enables precise prediction and is reliable under farm conditions. Before the onset of the study, a systematic literature research was conducted using the databases PubMed (www.pubmed.gov) and ScienceDirect (www.sciencedirect.com). As subject headings ‘calving’ AND ‘prediction’ and ‘cattle’ AND ‘calving behavior’ were used. The objective was to find articles in English or German which addressed signs of onset of calving. Specifically, we aimed to identify signs of imminent parturition (**SIP**) that are distinct, predictive and easily recognizable for farm staff. As a result of the literature research, we considered tail raising (Miedema et al., 2011), stepping (Richter and Ahlers, 1993), turning the head toward the abdomen (Jensen, 2012), clear vaginal discharge (Berglund et al., 1987), bloody vaginal discharge (Proudfoot et al., 2013) and lying lateral with abdominal contractions (Proudfoot et al., 2013) as meaningful and measurable criteria. We developed a score sheet of SIP on the basis of hourly observations.

According to a current review, the most accurate and sensitive prediction method is the combination of measuring relaxation of the pelvic ligaments and assays for progesterone and oestradiol-17β (Streyl et al., 2011; Saint-Dizier and Chastant-Maillard, 2015). Best results for predicting the absence of calving during the next 12-24 h were found by measuring the decrease in vaginal temperature and a combination of relaxation of the pelvic ligaments and teat filling (Burfeind et al., 2011; Streyl et al., 2011; Saint-Dizier and Chastant-Maillard, 2015). Our intention was to find a reliable prediction method which is feasible under farm conditions.

Therefore, the objective of the first study was to predict stage 2 of calving and to identify the value of signs of imminent parturition and changes in relaxation of pelvis ligaments and teat filling for calving prediction.

The results of this study were published in the Journal of Dairy Science:

K. Lange, C. Fischer-Tenhagen, W. Heuwieser. 2017. Predicting stage 2 of calving in Holstein-Friesian heifers. 100:4847–4856.

1.2 Effect of denaverine hydrochloride on calf vitality

The physiological adaption is an enormously important process for the newborn calf in order to cope with the extrauterine life (Kovacs et al., 2017). The process of calving and the first hours afterwards are determining for the prospective soundness of the calf (Barrier et al., 2013). Dystocia, meaning a difficult calving caused by prolonged calving or severe extraction (Mee, 2004), occurs more often in heifers compared to multiparous cows and has a negative influence on health and survival of calves (Meyer et al., 2000; Johanson and Berger, 2003; Lombard et al., 2007). Hence, good calving management is important for dam and calf.

Denaverine hydrochloride is a drug, that is approved to ease calving and can be administered to heifers during dystocia (Veyx-Pharma GmbH, 2009). Various approaches exist to measure calf vitality after parturition. One method that was originally developed for human neonates is the APGAR score (Apgar, 1953; Apgar et al., 1958). Therefore, heart rate, respiratory effort, reflex irritability, muscle tone and color of the mucous membranes were parameters that were evaluated to assess the condition of the newborn children. The APGAR score was modified in veterinary medicine (Mülling, 1977) and widely applied in clinical studies (Herfen and Bostedt, 1999; Probo et al., 2012; Vannucchi et al., 2015). The value of the APGAR score in identifying calves with limited vitality or an altered acid-base status is discussed controversially. When correlating clinic evaluation (i.e., APGAR score) of newborn vitality with different blood parameters (i.e., pH, base excess, carbon dioxide partial pressure) there is notable deviation (Herfen and Bostedt, 1999). Only 40.7 % of calves that were classified as vital by the APGAR score were classified as vital regarding the blood parameters (i.e., pH ≥ 7.2) (Herfen and Bostedt, 1999). Also, the authors report that with an increasing duration of calving, clinical scores indicated less vital calves than blood parameters (Herfen and Bostedt, 1999). In contrast to these results, Sorge et al. (2009) found a negative correlation between APGAR score and lactate concentration, which supports the predictive value of the former. More recently, Homerosky et al. (2017) examined blood gas disturbances, elevated l-Lactate concentration and tested muscle tonicity and other APGAR parameter in 77 calves. However, heart rate, respiratory rate and mucous membrane color (i.e., classical APGAR parameter) were not suitable for recognizing acidotic calves (Homerosky et al., 2017). Authors from different studies addressing canine neonatal vitality concluded that the APGAR score is the easiest and most reliable method for short-term survival prognosis under clinic surroundings (Veronesi, 2016).

Another method that is frequently used in evaluating neonatal vitality after calving is measuring the concentration of l-lactate (Burfeind and Heuwieser, 2012; Bleul and Gotz, 2013; Homerosky et al., 2017; Kovacs et al., 2017). Different handheld meters are available that enable a quick and reliable measuring result for an on farm analysis (Burfeind and Heuwieser, 2012; Karapinar et al., 2013). Lactate is built during anaerobic metabolism (Consitt et al., 2016). Frequently, calves suffer from a mixed respiratory-metabolic acidosis after parturition which can negatively affect their vitality or even cause death (Bleul and Gotz, 2013). As a plausible parameter we decided to measure lactate concentration directly after parturition.

Denaverine hydrochloride is a benzilacid derivative that is used in veterinary medicine to regulate myometrial contractions during birth (EMA, 1997). Denaverine hydrochloride (Sensiblex®, Veyx Pharma, Schwarzenborn, Germany) is approved in Germany to alleviate calving in heifers, to activate interrupted calvings and insufficient opening of the soft birth canal and the cervix (Veyx-Pharma GmbH, 2009). Other fields of application are facilitation of fetotomy and regulation of the intensity of labor contractions (Veyx-Pharma GmbH, 2009).

The objective of our second study was to measure the impact of DNH application on calf vitality, more precisely, APGAR score and lactate concentration. These data were collected during the second study whereas additionally the influence of DNH on calving ease was evaluated.

The results were recently published in Tierärztliche Praxis Ausgabe Großtiere/Nutztiere:

K. Lange, W. Heuwieser, C. Fischer-Tenhagen. 2018. Effect of denaverine hydrochloride application to heifers on the APGAR score and lactate concentration in newborn calves. 46:150-153.

1.3 Effects of denaverine hydrochloride on calving ease

Parturition is risky for mother and offspring and can result in a stress response, health problems, and maternal mortality (Mainau and Manteca, 2011). Regarding dystocia (i.e., a difficult calving, caused by a prolonged calving or severe extraction (Mee, 2004)) many adverse effects on dam and offspring are described. Dystocia is associated with an increased risk for metritis (Dubuc et al., 2010) and decreased fertility (Tenhagen et al., 2007; El-Tarabany, 2015). Calves that suffered from severe dystocia were at a higher risk of stillbirth (Lombard et al., 2007). Furthermore, they had a higher likelihood of treatment for respiratory or digestive diseases (Lombard et al., 2007). Barrier et al. (2012) observed dairy cows and calves in the first 3 hours after expulsion and found that assisted calves were less vigorous. In a study with 455 live born Holstein calves, lower passive immunity, an increased mortality rate and increased indicators of physiological stress were found in calves that experienced dystocia (Barrier et al., 2013).

Stress and pain management of farm animals has become an important issue with increasing interest of consumers (Chen et al., 2015). The standard approach of assessing stress in farm animals is the measurement of cortisol or corticosterone in blood plasma (Mormède et al., 2007). Another option is measuring these hormones in saliva, urine or feces avoiding stress through invasive sampling methods (Mormède et al., 2007). Heart rate variability as a non-invasive technique measuring the balance between sympathetic and vagal activity has been used for different types of stressors (von Borell et al., 2007). Sometimes a combination of concentration of cortisol and heart rate variability was used to examine acute stress responses (Nagel et al., 2016). Several other possibilities such as the composition of immune cells (Caroprese et al., 2010) or infrared thermography (Stewart et al., 2007) exist. As measuring glucocorticoids in blood plasma is considered the reference method (Mormède et al., 2007) and can be implemented under field conditions it was chosen to measure stress during parturition.

Literature dealing with pulling force during parturition is scarce. Becker et al. (2010) investigated the difference between alternate and simultaneous traction modes using dead calves in a biomechanical invitro model. In another study, the pulling force of 24 births was measured with a specially developed computer-controlled system (Wehrend et al., 2003). Pulling force was divided into light, medium and heavy. Peak values of 130 – 140 kp were measured.

Therefore, another objective of the second study was to evaluate the efficacy of denaverine hydrochloride treating heifers during parturition to ease calving.

The results of this study were recently published in the Journal of Dairy Science:

K. Lange, W. Heuwieser, C. Fischer-Tenhagen, 2019. Influence of denaverine hydrochloride on calving ease in Holstein-Friesian heifers. 102:5410-5418.

The papers are presented in the format outlined in the guide for authors of the respective journal.

2 RESEARCH PAPERS

2.1 Predicting stage 2 of calving in Holstein-Friesian heifers

2.2 Effect of denaverine hydrochloride application to heifers on the APGAR score and lactate concentration in newborn calves

2.3 Influence of denaverine hydrochloride on calving ease in Holstein-Friesian heifers

2.1 Predicting stage 2 of calving in Holstein-Friesian heifers

Predicting stage 2 of calving in Holstein-Friesian heifers

K. Lange*, C. Fischer-Tenhagen*, W. Heuwieser*

*Clinic for Animal Reproduction, Faculty of Veterinary Medicine, Freie Universität Berlin, Königsweg 65, 14163 Berlin, Germany

Published in:

Journal of Dairy Science, July 2017, Volume 100, Issue 6, Pages 4847–4856

Please find the original article via the following digital object identifier:

https://doi.org/10.3168/jds.2016-12024

2.1.1 **Abstract**

The objective of this study was to predict stage 2 of calving in Holstein-Friesian heifers. Interobserver reliability and predictive values of relevant signs of imminent parturition (i.e., tail raising, stepping, clear and bloody vaginal discharge, turning the head toward the abdomen, and lying lateral with abdominal contractions) were determined. In the first experiment 32 heifers were included. Three investigators participated as observers in the study. They walked through the precalving pen in pairs and observed pregnant heifers (≥267 d pregnant). Cohen's kappa results for the interobserver reliability were between 0.51 and 0.91. Thirty-seven Holstein-Friesian heifers were enrolled in the second experiment. Heifers were observed hourly for 24 h/d. Signs of imminent parturition that occurred were noted on a checklist. Compared with a precalving control period (4 d before calving), tail raising, clear vaginal discharge, and bloody vaginal discharge were more likely to occur during the last 24 h before calving. Two equations were built using the GENLINMIXED procedure to predict the hours until parturition. In version1, the absence or presence of each sign of imminent parturition except turning the head toward the abdomen was included. In version 2, hours until parturition were estimated with the factors days of gestation, tail raising, and clear vaginal discharge. Relaxation of the broad pelvic ligaments and teat filling were evaluated twice per day. Prediction of calving with these parameters was not satisfying (positive predictive values were between 35.1 and 72.7% depending on the day of gestation). The possibility of excluding calving for the next 12 h was considerably higher, ranging from 88.5 to 97.1%. These results indicate that predicting stage 2 of calving via direct observation of plausible signs is imprecise and therefore not recommendable.

Key words: calving, sign of parturition, prediction, dystocia

2.1.2 **Introduction**

Each calving is a crucial event in the reproductive cycle of a dairy cow and often a gateway for subsequent diseases. Optimal management and surveillance of calving, especially in heifers, are essential to prevent dystocia and stillbirth. This requires information on the exact time of calving. Parturition in cows may be divided into 3 stages (Noakes et al., 2001; Jackson, 2004). Stage 1 is characterized by opening of the cervix and the initiation of uterine contractions. The second stage starts with the dilation of the birth canal through the allantoic and amniotic sacs and ends with the expulsion of the fetus (Noakes et al., 2001). The release of the fetal membranes is the third stage of parturition (Noakes et al., 2001). The beginning of stage 2 is evident by the appearance of the amniotic sac outside the vulva (Schuenemann et al., 2011). The duration of stage 2 of calving is especially important for identifying problems in the calving process (Mee, 2004; Gundelach et al., 2009). The average time from the appearance of the amniotic sac to birth (i.e., the duration of stage 2 of calving) was 45.8 min for unassisted births in primiparous cows (Schuenemann et al., 2011). The median duration of stage 2 of calving was 42.7 min for unassisted heifers and 64.0 min for assisted heifers (Miedema et al., 2011a).

Although different approaches to predicting stage 2 of calving are practiced, no reliable method is available. Research has found that changes in behavior, such as restlessness, tail raising, and turning the head toward the abdomen (Miedema et al., 2011a; Barrier et al., 2012; Jensen, 2012), often occur 12 to 6 h before parturition. Moreover, vaginal discharge occurs (Schuenemann et al., 2011). Furthermore, the number of lying bouts increased during the last 6 h before parturition (Jensen, 2012).

Prior to the beginning of the study, a systematic literature search of clinical studies addressing signs of onset of calving was conducted. The databases PubMed (www.pubmed.gov) and ScienceDirect (www.sciencedirect.com) were used to find articles written in English or German about physiological and behavioral indicators of the onset of calving. The subject headings “calving AND “prediction” and “cattle” AND “calving behavior”were used. In a supplementary manual search, additional publications were recruited. The aim was to identify signs of imminent parturition (**SIP**) that are easy for farm personnel to recognize and predictive of the beginning of calving. Our focus was on developing a score sheet of SIP based on an hourly observation scheme. Finally, we considered tail raising (Figure 1; Miedema et al., 2011b), stepping (Richter and Ahlers, 1993), turning the head toward the abdomen (Jensen, 2012), clear vaginal discharge (Berglund et al., 1987), bloody vaginal discharge (Proudfoot et al., 2013), and lying lateral with abdominal contractions (Proudfoot et al., 2013) as plausible parameters for predicting the onset of calving (Table 1).

As a result of hormonal changes in preparation for calving, the broad pelvic ligaments relax, the udder enlarges, and teats fill up with colostrum (Berglund et al., 1987; Shah et al., 2006; Streyl et al., 2011). Monitoring cows antepartum is possible via direct observation, video surveillance, or the application of technical devices such as accelerometers (e.g., Ouellet etal., 2016), rumination sensors (e.g., Pahl et al., 2014), and temperature loggers (e.g., Burfeind et al., 2011). The clinical signs (i.e., relaxation of the broad pelvic ligaments, vaginal secretion, udder hyperplasia, udder edema, teat filling, tail relaxation, and vulva edema) alone and combined were evaluated to predict the time of parturition (Streyl et al., 2011). According to Streyl et al. (2011), the relaxation of the broad pelvic ligaments in combination with teat filling gave the best values for predicting either calving or no calving within 12 h, with a sensitivity ranging from 73.9 to 89.1 and a specificity ranging from 60.0 to 78.9 depending on the cut-off value. Ouellet et al. (2016) used 3 automated devices to measure vaginal temperature, rumination and lying time, and lying bouts to predict the onset of calving. Prediction of calving within the next 24 h was possible with a combination of the 4 parameters with sensitivity and specificity of 77%. Although monitoring the duration of the second stage of calving is especially important in preventing dystocia (Mee, 2004), the beginning of parturition is difficult to detect (Wehrendet al., 2006). In contrast, the absence of calving within the next 12 to 24 h can be accurately predicted by measuring a decrease in vaginal temperature and by the combination of pelvic ligament relaxation and teat filling (Burfeind et al., 2011; Saint-Dizier and Chastant-Maillard, 2015).

For efficient labor management it is important to know when to start monitoring pregnant heifers and how often observation is necessary. Therefore, the objective of our study was to predict stage 2 of calving via direct observation. Specifically, we set out to evaluatethe interobserver reliability for SIP and to determine the predictive values of SIP, changes in relaxation of the pelvic ligaments, and teat filling for calving.

2.1.3 ***Materials and Methods***

Animals and Housing

The study was conducted on a commercial dairy farm in Germany in October and November 2014. The farm milked 2,250 Holstein dairy cows with an average 305-d milk production of 10,346 kg.

All heifers were introduced to a precalving pen approximately 3 wk before the estimated calving date. The precalving pen was located in a transition management facility, in which heifers were housed from 3 wk before to 3 wk after calving. Group composition was dynamic, with new heifers being introduced into the pen once per week. The heifers were housed in a freestall barn with 36 cubicles covered with a layer of at least 15 cm of sand. The maximum number of heifers in the barn was 36. Within the pen the animals were able to move freely. When the calf's feet were visible outside the vulva, heifers were brought to an individual maternity pen with concrete topped with straw according to a standard operating procedure implemented by the farm. Within the pen the animals were able to move freely. Heifers were managed according to the guidelines set by the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (Hellmann and Radeloff, 2000).

Experimental Design: Experiment 1

In experiment 1, the interobserver reliability of SIP was tested. A total of 32 heifers were included. Three investigators participated in the study (all female; 1 veterinarian and 2 students of animal science). One investigator participated for 2 wk, and the others participated for 1 wk each. Before starting the experiments, the list of SIP was presented and discussed. A checklist with 6 items was developed for ticking off each recognized SIP. Initially, training rounds were performed. For training and validation purposes, the investigators walked in pairs through the precalving pen and recorded and discussed the presence or absence of SIP of the average 16 heifers in the pen. During the test rounds, 2 of the investigators walked through the precalving pen hourly and observed the heifers for possible SIP. Results of this phase were not used for the analyses of interobserver reliability. Approximately 8 observation rounds were performed each day. In each observation round the investigators walked and stood next to each other to ensure a similar angle of view but documented their observations independently (i.e., without communication) on the checklist. Each heifer was observed for 15 s using a stopwatch.

Statistical Analysis: Experiment 1

To determine the interobserver reliability of SIP, Cohen's kappa was calculated using SPSS (SPSS Inc., Chicago, IL). The observation results of observers 1 and 2 (wk 1) and the observation results of observers 1 and 3 (wk 2) were included. A Cohen's kappa value was determined for each SIP listed in Table 1 except for bloody vaginal discharge and lying lateral with abdominal contraction because of an insufficient frequency of appearance. If the kappa value was between 0.41 and 0.6, the strength of agreement was moderate. A kappa value between 0.61 and 0.8 and between 0.81 and 1 meant a substantial strength of agreement and an almost perfect strength of agreement, respectively (Landis and Koch, 1977).

Experimental Design: Experiment 2

Signs of Imminent Parturition. Six female investigators and 1 male investigator (5 veterinarians, 1 animal science student, and 1 veterinary technician) took part in experiment 2. All investigators were trained. The training implied discussing the list of SIP and implementing the checklist as described previously.

Thirty-seven clinically healthy Holstein-Friesian heifers 267 ± 4 d postinsemination were included in the study. Heifers were checked for SIP each hour 24 h/d. On average, 13 heifers were observed in each observation round. Observation was divided into 2 shifts (0800 to 2000 h and 2000 to 0800 h). The investigator walked through the pen, stopped behind a heifer, and observed the animal for 15 s. Observation rounds were conducted as quietly as possible to avoid any disturbance of the heifers. Each SIP that appeared was marked off on the checklist. Each SIP could be scored only once for a given observation round. As soon as abdominal contractions started, heifers were monitored every 10 min to determine the time when the amniotic sac or the feet became visible (i.e., stage 2 of calving). These extra observations were not included in the SIP protocol and were conducted to determine the time of appearance of the amniotic sac or the feet. When the amniotic sac or the calf's feet appeared outside the vulva, the heifer was brought into the maternity pen and the time of parturition was documented.

Relaxation of the Broad Pelvic Ligaments and Teat Filling. In addition to SIP, we examined relaxation of the pelvic ligaments and teat filling because these findings had the best predictive value for predicting calving within 12 h (Streyl et al., 2011). At 0800 and 2000 h, heifers were fixed in the head lockers for approximately 10 min. The same investigator evaluated the relaxation of the broad pelvic ligaments and teat filling on a 4-point scale according to Streyl et al. (2011) twice a day (Table 2). The relaxation of the broad pelvic ligaments was estimated by manual palpation. The investigator pressed the fingertips of both hands except the thumbs onto the caudal edge of both pelvic ligaments of each heifer. Teat filling was determined by visual inspection of the teat skin of both rear quarters; the teats were not manually investigated. The different grades of plication of the teat skin were evaluated. A totally plicated teat was indicative of an empty teat, and a teat skin without any plication was considered to be a teat filled with colostrum. Points for relaxation of the pelvic ligaments and teat filling were added and summarized as a parturition score. We categorized data for relaxation of the pelvic ligaments and teat filling according to the day of gestation for every heifer (i.e., from d 269 until d 276 of gestation). The category d 272 of gestation, for example, included all heifers that were examined from this day on until calving.

Statistical Analysis: Experiment 2

Data were analyzed using Microsoft Excel (version 2013; Microsoft Corp., Redmond, WA), SPSS for Windows (version 22.0; SPSS Inc.), and MedCalc for Windows (version 12.0.3.0; MedCalc Software bvba, Ostend, Belgium). The occurrence of SIP was visualized with graphs in Microsoft Excel. We focused on the period 24 h before calving (Figure 2).

Precalving Control Observation. Before building the prediction model, we wanted to identify possible predictors for an imminent calving. Therefore, we evaluated differences between the occurrences of SIP in late pregnancy and compared these with the last 24 h before the onset of stage 2 calving. A binary logistic regression model was used that included time as the input variable and the occurrence of SIP as outcome variables. A *P*-value threshold of 0.1 was used to select parameters for elimination from the model. For the precalving control period in late pregnancy (n = 30) we choose a period of 24 h on d 4 before calving (i.e., the time 96‑72 h before stage 2). Tail raising, turning the head toward the abdomen, stepping, clear vaginal discharge, and bloody vaginal discharge were included in the model, whereas lying lateral with abdominal contractions had to be excluded because it was not observed during the control period. In the last 24 h before calving, 35 heifers were included. Two heifers had to be excluded from the calculations because they were not completely observed during the last 24 h.

Prediction Model. A general linear mixed model was built to calculate the effect of tail raising, stepping, clear vaginal discharge, bloody vaginal discharge, lying lateral with abdominal contractions, and days of gestation on hours until parturition. In a first approach, SIP were included in the model as fixed factors and cows were included as random factors, respectively. The model was based on 6,539 observations on 37 heifers. On average, 176.7 observations/heifer were conducted.

In a second approach, days of gestation was additionally included as fixed factor. With the estimates of the general linear mixed model we set up an equation to predict the hours until parturition. Signs of imminent parturition were coded as 0 (not seen) or 1 (seen), respectively.

The model took the following form:

1) HuPij = b0 + (tail raisingij × b1) + (steppingij × b2) + (clear vaginal dischargeij × b3) + (bloody vaginal dischargeij × b4) + (lying lateral with abdominal contractionsij × b5) + ui + eij,

2) HuPij = b0 + (tail raisingij × b1) + (steppingij × b2) + (clear vaginal dischargeij × b3) + (bloody vaginal dischargeij × b4) + (lying lateral with abdominalcontractionsij × b5) + (day of gestationij × b6) + ui + eij,

where HuPij is hours until parturition for the ith cow and the jth number of measurements, b0 through b6 are parameter estimates, ui is the random effect, and eij is the residual error. The random effect and residual error were assumed to be normally distributed and independent from each other.

Observation Frequencies. A binary logistic regression model was used to calculate the effect of observation intervals on prediction of an imminent parturition within the next 6 h. The time interval was chosen because the occurrence of SIP increased approximately 6 h before stage 2. Thirty-seven calvings were included in the model. The occurrence of at least 2 out of 4 SIP (i.e., tail raising, clear vaginal discharge, bloody vaginal discharge, and lying lateral with abdominal contractions) at one observation time were considered as predictive of parturition. Odds ratios were calculated to compare hourly observation frequency with observation frequency each second hour until each sixth hour. Hourly observation frequency was the reference interval. The calculations were repeated, defining the 2-h observation interval as reference.

Relaxation of the Pelvic Ligaments and Teat Filling. To determine the usability of a given sign for predicting calving, a receiver operating characteristic analysis was conducted using MedCalc. Receiver operating characteristic analysis evaluates the predictive value of a diagnostic test. The area under the curve (**AUC**) determines the diagnostic performance. A value of 1 means that there is a perfect separation of the test values of both groups (i.e., the test identifies each case of calving), whereas a value of 0.5 indicates that the test is not able to distinguish between both groups (Zweig and Campbell, 1993; i.e., calving and no calving). According to Greiner et al. (2000), a test with an AUC between 0.7 and 0.9 is moderately accurate, whereas a test with an AUC between 0.9 and 1 is highly accurate. The AUC was calculated for relaxation of the pelvic ligaments, teat filling, and a combination of relaxation of the pelvic ligaments for which the scores of the pelvic ligaments were single and double weighted (Streyl et al., 2011). The classification was the outcome, meaning the presence or absence of calving.

2.1.4 ***Results***

Experiment 1

A total of 32 heifers were observed to determine the interobserver reliability in wk 1 and 2. Cohen’s kappa values were higher for all SIP in wk 2 compared with wk 1 (Table 3). Overall, reliability ranged between 0.507 and 0.912.

Experiment 2

Signs of Imminent Parturition. Amniotic sac or calves’ feet were seen 36.4 ± 33.9 min after the last regular observation of the SIP protocol. The occurrence of tail raising, bloody vaginal discharge, and lying lateral with abdominal contractions increased consistently within the last 4 observations before parturition (Figure 2). The first recognized SIP was clear vaginal discharge. Overall, it was observed 197 times out of 858 observations in the last 24 h before calving with hourly observation. In the second hour before stage 2, clear vaginal discharge was seen in 18 out of 37 heifers, which was the maximum occurrence of clear vaginal discharge in the last 24 h. As clear vaginal discharge was found at least 3 times in the last 24 h before stage 2, clear vaginal discharge alone was not considered to be a suitable predictor. An increase in bloody vaginal discharge was found from the fourth to the last hour before the amniotic sac or the calves’ feet were seen. Lying lateral with abdominal contractions occurred and increased from the third observation until stage 2. Tail raising increased consistently during the last 5 h until stage 2 and was the SIP that occurred most frequently in the last 6 h before parturition. Stepping and turning the head toward the abdomen did not occur often and did not increase before parturition. Therefore, both signs were disregarded.

Precalving Control Observation*.* A binary logistic regression analysis was conducted to determine the effect of observation time (i.e., late pregnancy vs. 24 h before stage 2) on the occurrence of SIP. Tail raising, clear vaginal discharge, and bloody vaginal discharge were more likely to occur 24 h before calving than in late pregnancy ($P \leq 0.001$; Table 4). Stepping had a tendency to be more frequent in the period before calving than 4 d before ($P = 0.073$). Between both groups there was no difference for turning the head toward the abdomen ($P = 0.331$). Six out of 10 heifers that stepped calved within the next 24 h (odds ratio = 1.696). Seven out of 10 heifers showing tail raising or clear vaginal discharge calved within 24 h. A heifer with bloody vaginal discharge had a 28.4 times greater probability of experiencing stage 2 during the next 24 h.

Calving Prediction. In the first approach, tail raising, stepping, clear and bloody vaginal discharge, and lying lateral with abdominal contractions showed a significant influence on time to parturition ($P < 0.05$; Table 5). Turning the head toward the abdomen showed a tendency to influence the outcome variable hours until parturition ($P = 0.061$) and was excluded from the final calculation. The occurrence of bloody vaginal discharge decreased hours until parturition approximately twice as much as clear vaginal discharge. Tail raising and stepping showed a similar influence on hours until parturition. A constant was calculated and each SIP that occurred decreased the constant. Finally, mean number of hours until parturition was calculated via multiplying the estimate of the GENLINMIXED model by 0 or 1 for each SIP and summing the constant:

Hours until parturition = 97.99 + (tail raising × −38.0) + (stepping × −37.65) + (clear vaginal discharge × −25.78) + (bloody vaginal discharge × −51.88) + (lying lateral with abdominal contractions × −30.52)

For example, a heifer seen with tail raising and bloody vaginal discharge during 1 observation needed 97.99 + (−38 × 1) + (−37.65 × 0) + (−25.78 × 0) + (−51.88 × 1) + (−30.52 × 0) = 8.1 h until parturition.

In the second approach, tail raising, clear vaginal discharge, and day of gestation showed a significant influence on hours until parturition ($P < 0.05$) as presented in Table 6.

Observation Frequencies. No difference in detecting imminent parturition was found between hourly observation and observation every 2 h ($P = 0.139$; Table 7). The probability of detecting stage 2 calving decreased when the interval between observations increased. The probability of detecting stage 2 of calving was 4.09, 9.17, and 11.63 times higher when observing heifers each hour compared with observing heifers each 3, 5, or 6 h, respectively. Considering a 2-h observation interval as a reference, detecting imminent parturition was significantly higher compared with a 3-, 4-, 5-, or 6-h observation interval, respectively.

Relaxation of the Pelvic Ligaments and Teat Filling. Heifers calved on average on d 275 ± 4.84 of gestation during the study (n = 37). The duration of pregnancy ranged from 267 d (n = 2) to 286 d (n = 1). The best results for the AUC were achieved when a combination of relaxed pelvic ligaments (double weighted) and teat filling (single weighted) was used. The AUC results ranged from 0.808 to 0.855 depending on the start of measurement (i.e., 269 to 276 d of gestation). An AUC of 0.8 determines that a calving heifer will have a higher parturition score than a noncalving one in 80% of the examinations (Zweig and Campbell, 1993).

Seventy-three percent of the heifers (n = 27) had a parturition score >5 at the last examination before calving. The maximum sum of sensitivity and specificity was estimated to identify a suitable cut-off value for the parturition score. The cut-off value was >5 except for examinations from d 276 of gestation until calving (Table 8).

Sensitivity and specificity were 68.97 and 87.91%, respectively, for predicting calving within 12 h for heifers (n = 29) examined from d 269 until calving; the cut-off value was >5 in 35.1% of the cases (Table 8). Negative predictive values were considerably higher. The possibility of predicting the absence of calving was 96.8% considering 29 heifers examined from d 269 of gestation until parturition, a cut-off value of >5, a 68.97% sensitivity, and a 87.91% specificity.

Table 1. List of signs of imminent parturition used in experiments 1 and 2

Sign of imminent parturition (study)	Description
Tail raising (Miedema et al., 2011)	The tail is raised from the base and held away from the body for at least 3 s. There is a distance of at least 5 cm from the distal end of the vulva to the tail.
Stepping (Richter et al., 1993)	Both hind legs are alternately raised and immediately put down again without changing the cow's position.
Turning the head toward the abdomen (Jensen, 2012)	Head is lifted and orientated toward the abdomen on stretched neck.
Clear vaginal discharge (Berglund et al., 1987)	At least 1 cm of clear discharge coming out of the vulva.
Bloody vaginal discharge (Proudfoot et al., 2013)	At least 1 cm of red-colored discharge coming out of the vulva.
Lying lateral with abdominal contractions (Proudfoot et al., 2013)	Cow is lying on the side or lying partially on the side with rhythmical movements of the abdominal muscles.

Table 2. Definition of the clinical signs relaxation of the broad pelvic ligaments and teat filling using a 4-point scale according to Streyl et al. (2011)

	Parturition Score			
Clinical signs	0	1	2	3
Relaxation of the broad pelvic ligaments	Firm, no – marginal relaxation (0 to 20%)	Mildly softened (up to 50%)	Totally softened, but palpable (up to 100%)	Totally softened, not palpable (100%)
Teat filling	Flaccid (None)	Slightly filled (~ 25%)	Moderately filled (~ 50%)	Completely filled (~ 100%)

Table 3. Results of the interobserver reliability in experiment 1

Parameter	Tail raising	Stepping	Turning the head	Clear vaginal discharge
Wk 1				
Cohen's kappa value	0.78	0.69	0.51	0.69
Total no. of observations	849	849	849	849
Observer agreement				
SIP[1] did not occur	806	821	788	749
SIP[1] occured	28	15	22	56
Observer disagreement	15	13	39	44
Wk 2				
Cohen's kappa value	0.89	0.77	0.91	0.84
Total no. of observations	670	670	670	670
Observer agreement				
SIP[1] did not occur	644	651	617	554
SIP[1] occured	21	12	45	88
Observer disagreement	5	7	8	28

[1]SIP = signs of imminent parturition.

Table 4. Binary logistic regression for signs of imminent parturition

Sign of imminent parturition	Odds ratio	95 % CI	*P*-value
Tail raising	2.506	1.428 - 4.398	0.001
Stepping	1.696	0.952 - 3.021	0.073
Head toward abdomen	1.382	0.720 - 2.656	0.331
Clear vaginal discharge	2.02	1.538 - 2.654	≤ 0.001
Bloody vaginal discharge	28.475	3.881 - 208.914	0.001

Table 5. Effect of signs of imminent parturition on hours until parturition

Variable	Estimate[1] Hours until Parturition	SE[2]	95% CI Lower	Upper	*P*-value
Constant	98.0				
Tail Raising					
No	Referent				
Yes	- 38.0	5.3	- 48.5	- 27.6	< 0.001
Stepping					
No	Referent				
Yes	- 37.7	6.4	- 50.1	- 25.2	< 0.001
Clear Vaginal Discharge					
No	Referent				
Yes	- 25.8	2.7	- 31.0	- 20.5	< 0.001
Bloody Vaginal Discharge					
No	Referent				
Yes	- 51.9	12.6	- 76.5	- 27.3	< 0.001
Lying lateral with abdominal contractions					
No	Referent				
Yes	- 30.5	13.0	- 56.1	- 5.0	0.019

[1] Model adjusted for the random effect of cow.

[2] Standard error of the estimate

Table 6. Effect of signs of imminent parturition and days of gestation on hours until parturition

Variable	Estimate[1] (h until parturition)	SE[2]	95% CI Lower	95% CI Upper	*P*-value
Constant	6,286.6				
Tail Raising					
No	Referent				
Yes	- 1.7	0.5	- 2.7	- 0.8	< 0.001
Clear Vaginal discharge					
No	Referent				
Yes	0.5	0.3	0.01	1.0	0.048
Days of gestation					
No	Referent				
Yes	-22.9	0.03	- 22.9	- 22.8	< 0.001

[1] Model adjusted for the random effect of cow.

[2] Standard error of the estimate

Table 7. Binary logistic regression model describing the effect of observation intervals on recognizing stage 2 of calving

Observation frequency	Estimate	Odds ratio	95 % CI	*P*-value
Each hour	Referent	Referent		
Each second hour	-0.721	0.486	0.187 - 1.264	0.139
Each third hour	-1.401	0.246	0.100 - 0.608	0.002
Each fourth hour	-1.866	0.155	0.064 - 0.375	≤ 0.01
Each fifth hour	-2.214	0.109	0.045 - 0.263	≤ 0.01
Each sixth hour	-2.449	0.086	0.036 - 0.207	≤ 0.01

Table 8. Results of a receiver operating characteristic analysis for predicting calving with the parameters pelvic ligaments and teat filling[1]

Days of gestation	No. of calvings	AUC	Cut-off value	Sensitivity	Specificity	+ PV	- PV
269	29	0.846*	>5	68.97	87.91	35.1	96.8
		(0.833-0.883)		(49.2-84.7)	(83.7-91.3)	(22.9-48.9)	(93.9-98.5)
270	27	0.855*	>5	74.07	86.94	36.4	97.1
		(0.809-0.893)		(53.7-88.9)	(82.3-90.7)	(23.8-50.4)	(94.1-98.8)
271	22	0.837*	>5	72.73	84.75	32.0	96.9
		(0.784-0.881)		(49.8-89.3)	(79.4-89.2)	(19.4-46.9)	(93.4-98.9)
272	23	0.838*	>5	73.91	83.68	35.4	96.4
		(0.782-0.885)		(51.6-89.8)	(77.6-88.6)	(22.0-50.7)	(92.3-98.7)
273	20	0.823*	>5	70.00	84.43	35.0	95.9
		(0.761-0.875)		(45.7-88.1)	(78.0-89.6)	(20.5-51.9)	(91.3-98.5)
274	20	0.814*	>5	70.00	82.03	37.8	94.6
		(0.742-0.873)		(45.7-88.1)	(74.3-88.3)	(22.5-55.2)	(88.6-98.0)
275	19	0.844*	>5	73.68	81.11	45.2	93.6
		(0.762-0.906)		(48.8-90.9)	(71.5-88.6)	(27.0-64.3)	(85.7-97.9)
276	15	0.808*	>6	53.33	94.74	72.7	88.5
		(0.698-0.891)		(26.6-78.7)	(85.4-98.9)	(39.0-94.0)	(77.8-95.3)

[1]Pelvic ligaments are double weighted. Values in parentheses are 95% CI. AUC = area under the curve; +PV = positive predictive value; −PV = negative predictive value.

*P < 0.05.

Figure 1. Tail raising.

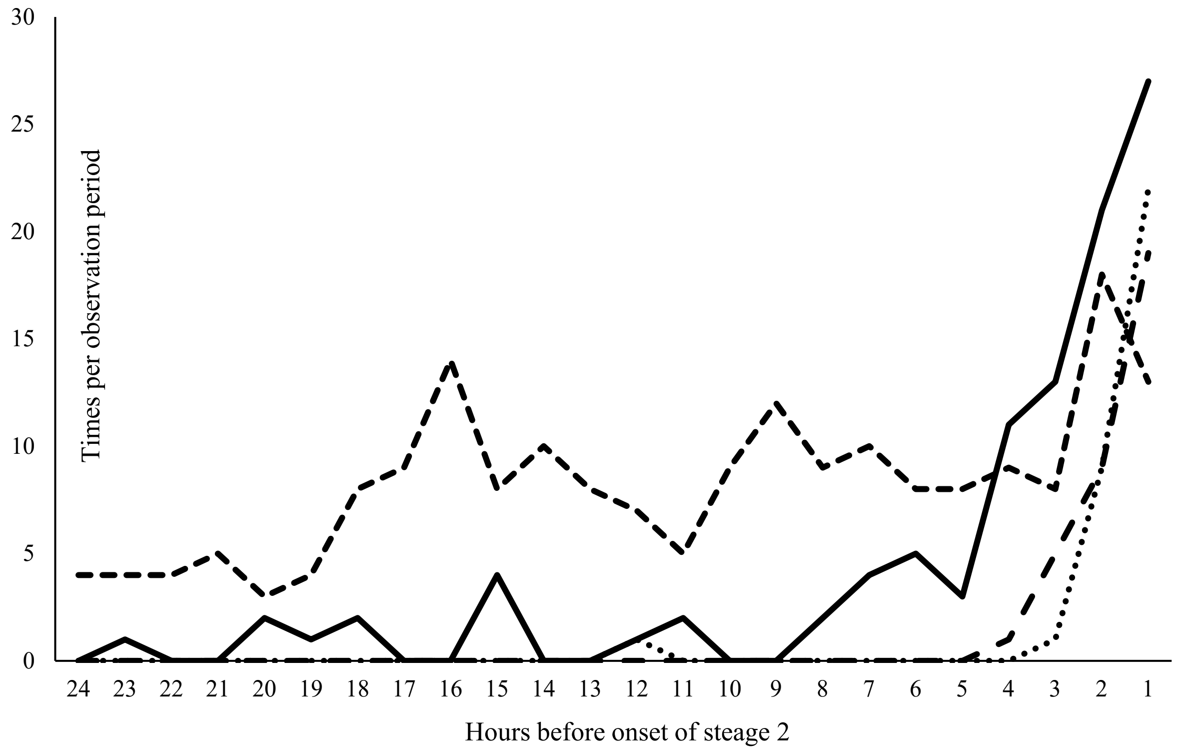


Figure 2. Occurrence of signs of imminent parturition: tail raising (solid line), clear vaginal discharge (short dashed line), bloody vaginal discharge (long dashed line), and lying lateral with abdominal contractions (dotted line) 24 h before stage 2.

2.1.5 **Discussion**

Recognizing landmarks of stage 2 of calving (e.g., the appearance of the amniotic sac or the calves' feet) is important in evaluating the progress of the expulsion of the fetus (Schuenemann et al., 2011). Prevalence of dystocia varies in different studies but is considerably higher in heifers compared with multiparous cows (Meyer et al., 2001; Lombard et al., 2007). Hence, predicting stage 2 of calving is of major importance, especially for primiparous dams.

Moving heifers during late stage 1 of calving has been shown to prolong the duration of stage 2 (Proudfoot et al., 2013). Farm staff, however, brought heifers to the calving pen during stage 2 of calving (i.e., as soon as the amniotic sac or the feet became visible). We consider this management practice to be critical because heifers showed some exploration behavior (sniffing, licking, vocalization) before lying down.

In our study, the interobserver reliability for the 4 SIP (i.e., tail raising, stepping, turning the head toward the abdomen, and clear vaginal discharge) was good or very good, except that for turning the head toward the abdomen in wk 1. This indicates that the list of SIP generated in combination with validation rounds is a useful tool for reaching good agreement for SIP if multiple observers are involved, which is usually the case on commercial dairy farms. Streyl et al. (2011) found similar kappa values for relaxation of the pelvic ligaments and teat filling (0.86 and 0.82, respectively).

In accordance with previous observations (Streyl et al., 2011), AUC results for the relaxation of the pelvic ligaments and teat filling were highest when the score of the pelvic ligaments was double weighted. Starting examination of relaxation of the pelvic ligaments and teat filling on d 270 of gestation led to an AUC (0.855) with sensitivity of 74.07% and specificity of 86.94%. However, 36.4% of the heifers were correctly diagnosed. Because these test characteristics were not satisfactory, we suggest using relaxation of the pelvic ligaments and teat filling to exclude the possibility of calving, confirming Streyl et al. (2011). Approximately 97% of the heifers that were diagnosed as not calving did not calve during the next 12 h. Knowing which heifer will not calve during the next 12 h facilitates monitoring and increases efficiency. Still, it is important to consider that the predictive value depends on the prevalence. In our data set prevalence varied between 8.6 and 20.8% depending on the day of gestation chosen as the starting point.

In contrast to our expectation, we recognized much variation in the appearance and time of occurrence of SIP. Tail raising, bloody vaginal discharge, and lying lateral with abdominal contractions first occurred approximately 9 h before stage 2. Miedema et al. (2011a) observed a significant increase in the frequency and duration of tail raising during the last 12 h before parturition. Although we concentrated on short observation periods regarding the 12 last observations until stage 2, we could also see a clear increase in tail raising and found a significant difference compared with our control period.

It appears to be useful to differentiate between clear and bloody vaginal discharge. There was a significant difference between the occurrence of vaginal discharge in the calving period in contrast to the control period (Table 4), but clear vaginal discharge was found intermittently several days before parturition in 31 of 35 heifers. Conversely, bloody vaginal discharge occurred in the calving group during the last 4 observations before stage 2 and was observed just once in the control group. Jensen (2012) described an increase in turning the head toward the abdomen in the last 2 h before calving. We could not confirm this finding, nor did a significant difference exist between the control and the calving period. This controversy is potentially attributable to the different observational methods. It is obvious that a behavioral sign with a duration of a few seconds (e.g., turning the head toward the abdomen) is better detected during continuous observation than during repeated observations lasting 15 s.

The recommendations for monitoring frequency of pregnant cows vary from 1 to 2 h (Gundelach et al., 2009; Schuenemann et al., 2011) to 3 to 6 h (Mee, 2004). Interestingly, our data showed a significant difference between hourly observation compared with 3- to 6-h observation intervals. There was no difference, however, between hourly observation compared with observation every second hour. Hence, we suggest observing pregnant heifers every 2 h for tail raising, clear and bloody vaginal discharge, and lying lateral with abdominal contractions. To observe the complete behavior repertoire for the time before parturition, cows have to be monitored continuously as implemented in several other studies (e.g., Miedema et al., 2011a; Barrier et al., 2012; Jensen, 2012). The scope of our study, however, was targeted toward field conditions. Therefore, we implemented direct observation periods at preset times to simulate common calving monitoring practices. Also, we limited observation time to 15 s/cow.

To our knowledge, an equation for the prediction of calving has not yet been developed. This is the first approach to using SIP to calculate time of expected calving on farms. Further research considering a larger sample size and different housing systems and breeds is warranted to increase robustness of the equation. After further expanding the database of SIP, we could imagine that this prediction model could be part of a technical application for farm personnel to easily and reliably predict stage 2 of calving.

2.1.6 ***Acknowledgements***

We gratefully thank the personnel of the dairy farm for their kind cooperation. Furthermore, we thank Veyx-Pharma and the staff of the Clinic for Animal Reproduction, Freie Universitat Berlin, Germany, for their support during the study. Katrin Lange was partially funded by Tiergyn e.V., Berlin.

2.1.7 ***References***

Barrier, A. C., M. J. Haskell, A. I. Macrae, and C. M. Dwyer. 2012. Parturition progress and behaviours in dairy cows with calving difficulty. Appl. Anim. Behav. Sci. 139 :209-217.

Berglund, B., J. Philipsson, and O. Danell. 1987. External Signs of Preparation for Calving and Course of Parturition in Swedish Dairy-Cattle Breeds. Anim. Reprod. Sci. 15:61-79.

Burfeind, O., V. S. Suthar, R. Voigtsberger, S. Bonk, and W. Heuwieser. 2011. Validity of prepartum changes in vaginal and rectal temperature to predict calving in dairy cows. J. Dairy Sci. 94:5053-5061.

Greiner, M., D. Pfeiffer, and R. D. Smith. 2000. Principles and practical application of the receiver-operating characteristic analysis for diagnostic tests. Prev. Vet. Med. 45:23-41.

Gundelach, Y., K. Essmeyer, M. K. Teltscher, and M. Hoedemaker. 2009. Risk factors for perinatal mortality in dairy cattle: cow and foetal factors, calving process. Theriogenology 71:901-909.

Hellmann, K. and I. Radeloff. 2000. International cooperation on harmonisation of technical requirements of veterinary medicinal products (vich). Brussels/Belgium. http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM052417.pdf

Jackson, P. G. G. 2004. Normal birth. Pages 1–12 in Handbook of VeterinaryObstetrics. 2nd ed. W. B. Saunders, Oxford, UK.

Jensen, M. B. 2012. Behaviour around the time of calving in dairy cows. Appl. Anim. Behav. Sci. 139:195-202.

Landis, J. R. and G. G. Koch. 1977. The Measurement of Observer Agreement for Categorical Data. Biometrics 33:159-174.

Lombard, J. E., F. B. Garry, S. M. Tomlinson, and L. P. Garber. 2007. Impacts of dystocia on health and survival of dairy calves. J. Dairy Sci. 90:1751–1760.

Mee, J. F. 2004. Managing the dairy cow at calving time. Vet. Clin. North Am. Food Anim. Pract. 20:521-546.

Meyer, C. L., P. J. Berger, K. J. Koehler, J. R. Thompson, and C. G. Sattler. 2001. Phenotypic trends in incidence of stillbirth for Holsteins in the United States. J. Dairy Sci. 84:515–523.

Miedema, H. M., M. S. Cockram, C. M. Dwyer, and A. I. Macrae. 2011a. Behavioural predictors of the start of normal and dystocic calving in dairy cows and heifers. Appl. Anim. Behav. Sci. 132:14-19.

Miedema, H. M., M. S. Cockram, C. M. Dwyer, and A. I. Macrae. 2011b. Changes in the behaviour of dairy cows during the 24 h before normal calving compared with behaviour during late pregnancy. Appl. Anim. Behav. Sci. 131:8-14.

Noakes, D. E., T. J. Parkinson, and G. C. W. England. 2001. Parturition and the care of parturient animals. Pages 155-187 in Arthur's Veterinary Reproduction and Obstetrics (Eighth Edition). W.B. Saunders, Oxford.

Ouellet, V., E. Vasseur, W. Heuwieser, O. Burfeind, X. Maldague, and E. Charbonneau. 2016. Evaluation of calving indicators measured by automated monitoring devices to predict the onset of calving in Holstein dairy cows. J. Dairy Sci. 99:1539-1548.

Pahl, C., E. Hartung, A. Grothmann, K. Mahlkow-Nerge, and A. Haeussermann. 2014. Rumination activity of dairy cows in the 24 hours before and after calving. J. Dairy Sci. 97:6935-6941.

Proudfoot, K. L., M. B. Jensen, P. M. Heegaard, and M. A. von Keyserlingk. 2013. Effect of moving dairy cows at different stages of labor on behavior during parturition. J. Dairy Sci. 96:1638-1646.

Richter, J. and D. Ahlers. 1993. Tiergeburtshilfe. Paul Parey, Berlin and Hamburg, Germany

Saint-Dizier, M. and S. Chastant-Maillard. 2015. Methods and on-farm devices to predict calving time in cattle. Vet. J. 205:349-356.

Schuenemann, G. M., I. Nieto, S. Bas, K. N. Galvao, and J. Workman. 2011. Assessment of calving progress and reference times for obstetric intervention during dystocia in Holstein dairy cows. J. Dairy Sci.94:5494-5501.

Shah, K. D., T. Nakao, and H. Kubota. 2006. Plasma estrone sulphate (E1S) and estradiol-17 beta (E-2 beta) profiles during pregnancy and their relationship with the relaxation of sacrosciatic ligament, and prediction of calving time in Holstein-Friesian cattle. Anim. Reprod. Sci. 95:38-53.

Streyl, D., C. Sauter-Louis, A. Braunert, D. Lange, F. Weber, and H. Zerbe. 2011. Establishment of a standard operating procedure for predicting the time of calving in cattle. J. Vet. Sci. 12:177-185.

Wehrend, A., E. Hofmann, K. Failing, and H. Bostedt. 2006. Behaviour during the first stage of labour in cattle: Influence of parity and dystocia. Appl. Anim. Behav. Sci. 100:164-170.

Zweig, M. H. and G. Campbell. 1993. Receiver Operating Characteristic (Roc) Plots - a Fundamental Evaluation Tool in Clinical Medicine. Clin Chem 39:1589-1589.

2.2 Effect of denaverine hydrochloride application to heifers on the APGAR score and lactate concentration in newborn calves

Effect of denaverine hydrochloride application to heifers on the APGAR score and lactate concentration in newborn calves

K. Lange*, W. Heuwieser*, C. Fischer-Tenhagen*

*Clinic for Animal Reproduction, Faculty of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany

Published in:

Tierärztliche Praxis Ausgabe G: Großtiere/Nutztiere, June 2018, Volume 46, Issue 3, Pages 150-153

Please find the original article via the following digital object identifier:

https://doi.org/10.15653/TPG-170844

2.2.1 **Summary**

Objective: The aim of this study was to evaluate the influence of Denaverine hydrochloride (DNH) in heifers on calf vitality. ***Material and methods:*** A total of 83 calvings with 38 female and 45 male calves were included in the study. Thirty minutes after onset of stage 2 of calving, 400 mg DNH or placebo (0.9% NaCl) were administered subcutaneously. If the calving procedure was not completed after 60 minutes, an extraction was conducted and pulling force was measured by using a digital force gauge. Directly after parturition, vitality of calves was evaluated using a modified APGAR score. Additionally, lactate concentration in blood from Vena auricularis was measured with a handheld measuring device (lactate scout). ***Results:*** No effect of treatment was observed on APGAR score and lactate concentration. ***Clinical relevance:*** Denaverine hydrochloride is a regularly used substance in obstetrics in veterinary medicine in many European countries. We could not confirm our hypotheses that treating heifers with DNH has a positive effect on calf vitality evaluated by APGAR score and lactate concentration in blood.

Keywords
Cattle - calving - lactate – vitality

2.2.2 **Zusammenfassung**

Ziel: der Studie war, den Effekt einer Behandlung von Färsen mit Denaverinhydrochlorid auf die Vitalität der geborenen Kälber zu testen. ***Material und Methoden:*** Die Auswertung umfasste 83 Färsengeburten, bei denen 38 weibliche und 45 männliche Kälber geboren wurden. Eine halbe Stunde nach Beginn der Aufweitungsphase wurden dem Muttertier randomisiert 400 mg Denaverinhydrochlorid oder ein Plazebo subkutan appliziert. War das Kalb nach 60 Minuten nicht geboren, erfolgte eine Zughilfe mit Messung der benötigten Zugkraft. Unmittelbar nach der Geburt wurde die Vitalität des Kalbes mit einem modifizierten APGAR-Score bewertet und in einer Blutprobe aus der Vena auricularis mit einem Handmessgerät die Laktatkonzentration bestimmt. ***Ergebnisse***: Es wurde kein Effekt der Behandlung auf APGAR-Score und Laktatkonzentration der Kälber im Blut festgestellt. ***Schlussfolgerung und klinische Relevanz:*** Denaverinhydrochlorid ist eine in vielen Ländern der EU in der Geburtshilfe regelmäßig eingesetzte Substanz. Wir konnten die Hypothese, dass die Behandlung von Färsen mit DNH einen positiven Effekt auf die Vitalität der Kälber hat, gemessen am APGAR-Score und der Laktatkonzentration im Blut, nicht beweisen.

Schlüsselwörter

Rind - Geburt - Laktat – Vitalität

2.2.3 **Introduction**

Several mechanisms are necessary for newborn calves to adapt from the intra- to the extrauterine life. The fetrus needs an adequate oxygen saturation of the blood and has to regulate the acid-base balance (14). Forceful extraction of calves is the most important cause of calving traumata such as rib and vertebral fractures in the neonate (19). Calves born with dystocia showed reduced vitality in the first 3 hours after birth (4). Severe dystocia is also associated with increased stillbirth rate and loss of calves up to 30 days of age (15).

Denaverine hydrochloride (DNH) is a benzyl acid derivate with spasmolytic and analgesic effects (12, 13) and approved in Germany for cattle with the indication to regulate labor contractions and promotes dilation of the soft birth canal (Sensiblex®, Veyx-Pharma GmbH, Schwarzenborn, Germany). A positive effect of DNH has been described to reduce the number of assisted calvings and to improve uterine health and reproductive performance in next lactation (22). The influence on calf vitality has not been investigated yet.

Several methods exist to score calf vitality after parturition. The APGAR score (2) was originally established to evaluate the chance of survival of newborn babies. This score is still part of routine diagnostic of newborn babies. It is generally performed between 1 and 5 minutes after birth and may be repeated later for newborns with low scores. The 8-point score was modified to assess calf vitality (17) based on respiratory rate, color of the mucous membranes of the gums, spontaneous movements of the head and direct reaction to manipulation (Table 1). Each criterion is rated as absent (0), weak (1) or strong (19) respectively. Calf scores of 0–3 points are rated as depressed, scores of 4–6 as endangered for death and scores of 7–8 as vital (2, 17, 20).

Concentration of lactate in blood is another method to identify compromised calves (20). Lactate is produced during anaerobic glycolysis. The end-product of glycolysis, i. e., pyruvate is converted via lactate dehydrogenase into lactate during anaerobic metabolism (7). The main sources of lactate are the skeletal muscles and the gut (3). Local or systemic ischemia can be identified through an increased concentration of blood lactate (11, 1). Calves that were born with assistance (8) or experienced a long duration of parturition (i. e., > 6 hours after the rupture of the allantoic sac) (5), had significantly higher concentrations of plasma lactate.

The objectives of our study were to evaluate the effect of DNH on the vitality and the concentration of lactate in blood of newborn calves.

2.2.4 **Material and Methods**

Eighty-three Holstein-Friesian heifers (757 ± 71 days of age, 670 ± 55 kg body weight) were enrolled in the experiment. The calving pen was a free stall facility with deep straw bedding. Calving monitoring was conducted via direct observation and video surveillance. Thirty minutes after at least one foot of a calf was visible outside the vulva, DNH (Sensiblex®, Veyx-Pharma GmbH, Schwarzenborn, Germany, n = 41) or placebo (NaCl 0,9% solution, PLA, n = 42) was administered subcutaneously to the heifer. The assignment to the treatment group was randomized and the administration blinded. If the calf was not born 30 minutes after administration, calving assistance was provided. A standing heifer was casted down with a rope to ensure that assistance was conducted while the heifer was lying in a sternal position. Pulling force was measured with a digital force gauge. The vitality of the newborn calf was scored with the modified APGAR score (9, 17,18) directly after parturition. For each criterion 0, 1 or 2 score points were given. A maximum of 8 points described a fully vital calf while 0 points indicated a stillborn calf. Concentration of lactate in blood was measured with a handheld device (Lactate Scout, SensLab GmbH, Leipzig, Germany), and a drop of blood from V. auricularis was gained using a 21-gauge needle (Sterican®, B. Braun, Melsungen, Germany). The device was evaluated for animal-side detection of lactate concentrations of cows and calves (6).

Data were entered into Excel (Microsoft Corp., Redmond, WA) spreadsheets and analyzed using SPSS for Windows (version 22.0, SPSS Inc., IBM, Ehningen, Germany). The effect of treatment on the vitality of the calves (i. e., APGAR score) was estimated utilizing a generalized linear model for ordinal data. A generalized linear mixed model was used to determine the effect of treatment on the lactate concentration.

2.2.5 **Results**

Denaverine hydrochloride and PLA were administered to 41 and 42 heifers, respectively. Thirty-six heifers calved without assistance while 47 heifers required calving assistance, since the calf was not born 60 minutes after at least one foot was visible in the vulva. Eighty-three calves were born, of which 38 were female and 45 male. The calves weighed 41.0 ± 4.6 kg on average.

For two calves the APGAR score was not estimated, thus 81 calves were included in the analysis. One calf was born dead (APGAR 0). Fifty-eight calves were born with an APGAR of ≥ 6. An effect of DNH on calf vitality was not found ($p = 0.084$).

Lactate was measured on average 6 ± 3 minutes after calving. Seventy-one blood samples were analyzed for lactate concentration. For DNH and PLA, lactate concentration ranged from 3.1–18.8 mmol/l for assisted and unassisted calving’s (n = 71). Mean concentration was 10.6 ± 3.9 mmol/l for calves born from heifers treated with DNH and 10.1 ± 3.6 mmol/l for calves born from heifers treated with PLA, respectively. Denaverine hydrochloride did not affect lactate concentration of calves (p = 0.613).

Parameter	Score 0	Score 1	Score 2
respiration	absent	weak	strong, regular
mucous membrane	pale	cyanotic	red
reflex occurrence	absent	reduced	immediate
spontaneous movement, muscle tension	absent	reduced	present

Table 1 Modified APGAR-Score.

Tab. 1 Modifizierter APGAR-Score

2.2.6 **Discussion**

This study was conducted to evaluate the influence of DNH in heifers on calf vitality after parturition. We could not demonstrate any influence of a DNH treatment on APGAR score or lactate concentration. A prolonged or assisted calving can produce birth stress and lead to injury, inflammation, hypoxia, acidosis, pain and maladaptation in the newborn calf causing reduced vitality (18). An index-based APGAR score is established in human infants after delivery but has failed to be adopted by cattle producers and veterinarians outside research settings (10). The authors speculated that the complexity of the score and missing recommendations for intervention are reasons for its lack of popularity. Acidotic calves could not be identified with APGAR criteria (i. e., heart rate, respiratory rate, color of mucous membrane), but tongue withdrawal (as a test of muscle tonicity), calving ease and parity were criteria associated with a decreased blood pH and an increased lactate concentration (10). In our study, neither the APGAR score nor lactate concentration in blood differed between the treatment groups. Zobel and Taponen (22) studied the influence of DNH and Carbetocin (i.e., a long-acting oxytocin) on several parameters during parturition. Furthermore, the authors reported fewer cases of dystocia and less animals requiring episiotomy or suffering from postpartum birth canal lesions. Calf mortality did not differ between the treatment groups (22). One explanation for the lack of a difference in our study between both groups could be that the process of calving itself is enormously stressful for the neonate per se so that a potential influence of DNH on the APGAR score or lactate concentration is superposed. Furthermore, time for spontaneous calving was limited to 60 minutes which might have confounded results of vitality.

Higher lactate concentrations were found for calves that were exposed to assisted calvings, compared to unassisted calvings (8, 20, 21). Mean lactate concentrations reported by Vermorel et al. (21) were comparable to the results of our study for normal (5.5 ± 4.2 mmol/l) and for assisted calving (11.3 ± 4.7 mmol/l). In another study lactate concentration depended on breed (Friesian calves: 4.9 ± 0.3 mmol/l, Angus-cross calves: 7.5 ± 0.5 mmol/l) and was lower than our values (8). This could be attributed to a quicker blood sampling and direct measuring method with the lactate scout. Furthermore, the authors used a study population of cows and heifers. As we only focused on heifers, prevalence of dystocia was supposed to be higher (15, 16) resulting in higher lactate concentrations.

Regarding the process of calving, less pulling force was needed for heifers in the DNH group (unpublished data) thus proving the beneficial effects of DNH, but no difference was found in the increase of cortisol after parturition between the treatment groups. Further research is required to evaluate if other parameters exist that better identify the influence of DNH treatment on calf vitality.

Conclusion for practice

APGAR score of newborn calf is not influenced by treating heifers with denaverine hydrochloride.

2.2.7 **Acknowledgements**

We greatly appreciate the kind cooperation of the collaborating dairy farm. We thank Veyx-Pharma GmbH for partly funding the study and all staff members of the Clinic for Animal Reproduction, Freie Universität Berlin (Berlin, Germany) for the support during the study. Katrin Lange was funded in part by a scholarship of Tiergyn e. V. (Berlin, Germany).

Conflict of interest

Although Veyx-Pharma partly funded this study, the authors declare that there is no conflict of interests.

2.2.8 **References**

1. Andersen LW, Mackenhauer J, Roberts JC, Berg KM, Cocchi MN, Donnino MW. Etiology and therapeutic approach to elevated lactate levels. Mayo Clin Proc 2013; 88 (10) 1127-1140.
2. Apgar V, Holaday DA, James LS, Weisbrot IM, Berrien C. Evaluation of the newborn infant; second report. JAMA 1958; 168: 1985-1988.
3. Arieff AI, Graf H. Pathophysiology of type A hypoxic lactic acidosis in dogs. Am J Physiol 1987; 253: E271-276.
4. Barrier AC, Ruelle E, Haskell MJ, Dwyer CM. Effect of a difficult calving on the vigour of the calf, the onset of maternal behaviour, and some beha-vioural indicators of pain in the dam. Prev Vet Med 2012; 103 (04) 248-256.
5. Bleul U, Gotz E. The effect of lactic acidosis on the generation and compen-sation of mixed respiratory-metabolic acidosis in neonatal calves. Vet Rec 2013; 172: 528.
6. Burfeind O, Heuwieser W. Validation of handheld meters to measure blood L-lactate concentration in dairy cows and calves. J Dairy Sci 2012; 95: 6449-6456.
7. Consitt LA, Saxena G, Saneda A, Houmard JA. Age-related impairments in skeletal muscle PDH phosphorylation and plasma lactate are indicative of metabolic inflexibility and the effects of exercise training. Am J Physiol En-docrinol Metab 2016; 311 (01) E145-56.
8. Diesch TJ, Mellor DJ, Stafford KJ, Ward RN. The physiological and physical status of single calves at birth in a dairy herd in New Zealand. N Z Vet J 2004; 52: 250-255.
9. Herfen K, Bostedt H. Correlation between clinical and laboratory diag-nostic evaluation of the vitality of newborn calves under particular consi-deration of length and type of parturition. Wien Tierarztl Monatsschr 1999; 86: 255-261.
10. Homerosky ER, Caulkett NA, Timsit E, Pajor EA, Kastelic JP, Windeyer MC. Clinical indicators of blood gas disturbances, elevated L-lactate con-centration and other abnormal blood parameters in newborn beef calves. Vet J 2017; 219: 49-57.
11. Hughes D, Rozanski ER, Shofer FS, Laster LL, Drobatz KJ. Effect of sam-pling site, repeated sampling, pH, and PCO2 on plasma lactate concentra-tion in healthy dogs. Am J Vet Res 1999; 60: 521-524.
12. Hüller H, Scheler W. Zur Pharmakologie einer Serie neuer Benzilsäurederi-vate. Acta biologica et medica Germanica 1963; 11.

13. Hüller H. Pharmakologie und Klinik von O-(2-Äthylbutoxy)-benzil-säure-2-dimethylaminoäthylesterhydrochlorid (= Spasmalgan). Zentralbl Pharm 1970; 109.

14. Kasari TR. Physiologic mechanisms of adaptation in the fetal calf at birth. Vet Clin North Am Food Anim Pract 1994; 10 (01) 127-136.

15. Lombard JE, Garry FB, Tomlinson SM, Garber LP. Impacts of dystocia on health and survival of dairy calves. J Dairy Sci 2007; 90 (04) 1751-1760.

16. Meyer CL, Berger PJ, Koehler KJ, Thompson JR, Sattler CG. Phenotypic trends in incidence of stillbirth for Holsteins in the United States. J Dairy Sci 2001; 84: 515-523.

17. Mülling M. Asphyxie des neugeborenen Kalbes. Prakt Tierarzt 1977; 58: 78-80.

18. Murray CF, Leslie KE. Newborn calf vitality: Risk factors, characteristics, assessment, resulting outcomes and strategies for improvement. Vet J 2013; 198: 322-328.

19. Schuijt G. Iatrogenic fractures of ribs and vertebrae during delivery in peri-natally dying calves: 235 cases (1978-1988). J Am Vet Med Assoc 1990; 197: 1196-202.

20. Sorge U, Kelton D, Staufenbiel R. Neonatal blood lactate concentration and calf morbidity. Vet Rec 2009; 164: 533-534.

21. Vermorel M, Dardillat C, Vernet J, Saido Demigne C. Energy metabolism and thermoregulation in the newborn calf. Ann Rech Vet 1983; 14: 382-389.

22. Zobel R, Taponen J. Denaverine hydrochloride and carbetocin increased welfare during and after parturition and enhanced subsequent fertility in cattle. J Dairy Sci 2014; 97 (06) 3570-3577.

2.3 Influence of denaverine hydrochloride on calving ease in Holstein-Friesian heifers

K. Lange*, W. Heuwieser*, C. Fischer-Tenhagen*

*Clinic for Animal Reproduction, Faculty of Veterinary Medicine, Freie Universität Berlin, Königsweg 65, 14163 Berlin, Germany

Published in:

Journal of Dairy Science, March 2019, Volume 102, Issue 6, Pages 5410-5418

Please find the original article via the following digital object identifier:

https://doi.org/10.3168/jds.2018-14930

3.1.1 **Abstract**

Calving is assumed to be an exhausting and painful event. A drug that eases the calving procedure and alleviates pain would help cows, especially those suffering from dystocia. In a randomized, controlled, and blinded trial we measured the effect of denaverine hydrochloride on physical and physiological calving parameters. Eighty-three Holstein Friesian heifers were included in the analysis. Pulling force was measured using a digital force gauge interposed between the calf and a mechanical calf puller. The concentration of cortisol was measured in serum before and after parturition. There was no effect of treatment group on calving modality (i.e., spontaneous vs assisted calving), duration of calving, and cortisol concentration. The area under the curve of pulling force x time (n=44), however, was significantly smaller in the treatment group compared to the placebo group. Also, duration of calving assistance was numerically shorter in the treatment group compared with the placebo group. The results provide evidence that calving ease can be influenced by denaverine hydrochloride during calving assistance.

Key words: pulling force, calving ease, pain, obstetric

3.1.2 **Introduction**

Optimal calving management is important to control dystocia in dairy cows (Mee, 2008). Dystocia, defined as difficult calving, caused by a prolonged calving or severe assisted extraction (Mee, 2004) is associated with negative consequences for the dam [i.e., increased risk for metritis (Dubuc et al., 2010), decreased fertility (Tenhagen et al., 2007), and economic losses (Dematawena and Berger, 1997)] and for the calf (e.g., stillbirth, respiratory and digestive disease; Lombard et al., 2007). Calving is described as a painful procedure (Mainau and Manteca, 2011). A survey answered by 641 cattle practitioners in the United Kingdom, demonstrated that dystocia is being perceived as one of the most painful conditions in adult cattle (Huxley and Whay, 2006). Heifers especially suffer due to a prolonged calving duration with higher effort (Noakes et al., 2001) and a higher incidence of dystocia in contrast to multiparous cows (Funnell and Hilton, 2016). In a recent survey, animal welfare was the primary issue raised by consumers regarding dairy farming (Cardoso et al., 2016). According to US and German Animal Welfare Acts it is mandatory to avoid pain, suffering and harm in animals (BMJV, 2017, USDA, 2017). For farm animals the “5 freedoms” were developed as objectives for animal welfare (Farm Animal Welfare Council, 1993).

Pain evaluation in cattle is challenging and has been studied with different approaches (Dyer et al., 2007; Heinrich et al., 2010; Huber et al., 2013). Recently, the first evidence for pain evaluation based on head and ear position, attention, response to approach, facial expressions, and back position was presented (Gleerup et al., 2015). In an experiment with 96 cows, the pain score of diseased cows decreased after treatment with an analgesic, whereas the score of control animals remained constant after treatment (Gleerup et al., 2015). In farm animals, physiological reactions such as the release of glucocorticoids (Mormède et al., 2007), the change of heart rate variability (von Borell et al., 2007; Nagel et al., 2016b), or the varying composition of immune cells (Caroprese et al., 2010) were used to evaluate stress. For acute stress responses, cortisol concentrations in blood plasma, serum (Negrao et al., 2004; Civelek et al., 2008; Saco et al., 2008), or saliva (Barrier et al., 2013; Nagel et al., 2016a) have been measured in farm animals. Cortisol concentration in blood serum was measured during this study to measure the heifers' stress during parturition. Cortisol was measured in serum as blood is easy to obtain. Furthermore, cortisol is an established substance for stress measurement in farm animals (Mormède et al., 2007; Huzzey et al., 2011). Alternative materials such as urine, milk, saliva, and feces were discussed beforehand but disregarded. Sampling techniques would have required more handling or more frequent sampling intervals (Möstl and Palme, 2002; Mormède et al., 2007).

Substance P is a neuropeptide that is included in the regulation of pain in animals (Coetzee, 2011). In veterinary medicine, it is determined in blood plasma to examine pain reactions in presumably painful procedures such as castrations or electroejaculation (Coetzee et al., 2008; Whitlock et al., 2012; Dockweiler et al., 2013). As this parameter is less common than cortisol measurement, we decided to examine the latter due to reasons of comparability.

Prevalence of calving assistance ranges between 10 % and over 50 % worldwide (Mee, 2008). Lombard et al. (2007) analyzed over 7,000 calvings from 3 Colorado dairy farms and described that over 50 % of the calves born to primiparous dams were delivered with assistance. Assisted calvings can result in vulvovaginal laceration and retained placenta (Kovács et al., 2016). Vulvovaginal lacerations occurred in 18.8 and 80 % of the cows that experienced appropriately timed assistance and premature assistance (i.e., < 65 min after the appearance of the fetal hooves), respectively. In contrast, vulvovaginal lacerations were found in none of the cows that calved spontaneously in a group pen and in 9.5 % of the cows that calved spontaneously in an individual pen (Kovács et al., 2016). Calving difficulty may negatively influence milk production and saleable milk yields (Barrier and Haskell, 2011). Furthermore, calves that experience dystocia suffer from higher stress, reduced transfer of passive immunity and higher morbidity and mortality (Tenhagen et al., 2007; Barrier et al., 2013).

Sensiblex (Denaverine hydrochloride, **DNH;** Veyx-Pharma, Schwarzenborn, Germany) is currently approved to treat dystocia in dairy cows and dogs in several EU (e.g., Bulgaria, France, Germany, Ireland, Italy, Poland, Spain and United Kingdom) and non-EU countries (e.g., Egypt, Mexico, Russia, Turkey, and Ukraine). This drug improves the elasticity of the soft birth canal and regulates labor contractions (Veyx-Pharma GmbH, 2009). In addition, a certain analgesic effect was described in mice (Hüller, 1970). In a recent study, a positive effect of a combination of DNH (400 mg i.m. once or twice) and 0.35 mg oxytocin in a long-acting formulation (Carbetocin, i.m. once or twice) on calving ease and postpartum reproductive health was found (Zobel and Taponen, 2014). Unfortunately, the study was not blinded and the manual estimation of the opening of the vulva and cervix must be considered confounded due to subjectivity. Efficacy of DNH alone has not been evaluated yet. DNH is approved to ease calving specifically in heifers. Dystocia occurs more frequently in primiparous dams compared with multiparous dams (Lombard et al., 2007); primiparous dams were therefore chosen as the study population. Furthermore, we aimed to have a consistent study population.

Interestingly, science-based information on measuring pulling force during calving assistance is scarce. Becker et al. (2010) developed an in vitro model to measure and compare forces during calving assistance with different pulling techniques. Until the entry of the elbows into the pelvis, maximum force on one leg measured during simultaneous traction was 431 ± 127 N. During alternate traction, maximum values were 411 ± 86 N with a difference in traction of 5 cm and 341 ± 106 N with a difference of 10 cm between both legs. The latter value differed from the measured force of simultaneous traction ($P \leq 0.01$). In another in vivo study, pulling force was measured in 24 calvings using a mechanical calf puller and a computer-controlled system (Wehrend et al., 2003). The authors categorized the pulling forces into light (~ 490 N, duration: 41 ± 21 s), moderately heavy (~ 784 – 981 N, duration: 86 ± 22 s), and heavy (~ 981-1,177 N, duration: 268 ± 117 s) pulling force. In 2 cases lesions were found after moderately heavy force, and in 5 cases lesions occurred after heavy pulling force was applied. Maximum values of up to 1,471 N were recorded.

To our knowledge, a randomized controlled trial investigating the efficacy of DNH considering calving ease (i.e., pulling force and duration of parturition) has not yet been conducted. Therefore, the objective of this study was to evaluate the efficacy of DNH in heifers during parturition. Specifically, we set out to evaluate pulling force in parturitions to determine duration of parturitions and to compare cortisol concentration before and after parturition in heifers treated with DNH and placebo.

3.1.3 **Materials and Methods**

Animals and Housing

The study was conducted on a commercial dairy farm in Sachsen-Anhalt, Germany, between June and November 2015. A total of 120 heifers were enrolled in the study on day 267 ± 2 post insemination. Overall, 37 heifers (30.8 %) were excluded due to abnormal presentations, positions or postures during parturition and oversized calves (n = 8) or for not complying with the timeline of the study protocol (n = 29). The timeline was abandoned when signs of premature expulsion of the placenta (i.e., expulsion of the placenta concurrently with the expulsion of the fetus) were visible and calves showed a considerably blue colored tongue and clear lingual edema, which necessitated a faster intervention to prevent risks for calves.

The remaining 83 Holstein-Friesian heifers (age: 757 ± 71 d; BW: 670 ± 55 kg) were included in the analyses. Heifers in the last month of pregnancy were housed in a freestall barn with slatted floors and stalls bedded with recycled manure solids. Heifers were fed a TMR. The rations were formulated to meet or exceed NRC (2001) requirements. Feed was delivered over a conveyer belt system 10 times per day. Approximately 3 to 5 d before the estimated calving date, heifers were brought to a freestall facility with deep bedded straw serving as a calving pen. A TMR was fed once a day at approximately 0700 h and pushed up 8 times per day. All cows had access to water ad libitum.

Experimental Design

Heifers in the precalving pen were enrolled on day 267 ± 2 post insemination. On this day a clinical examination was performed, including pulse at the A. coccygea mediana, respiratory rate, and rectal temperature. Furthermore, body condition was scored and BW was measured with a weight measuring tape (Topagrar, Landwirtschaftsverlag GmbH, Münster, Germany). This examination was conducted to ensure that heifers were without pathological findings.

Blood sampling was conducted at enrollment and at weekly intervals. For analyses, the last measurement before calving (i.e., before the heifer was brought to the calving pen) was compared with the measurement directly after calving. Blood samples were drawn from the coccygeal vein with a Vacutainer system (Vacuette 8-mL Serum Beads Clot Activator, Greiner Bio-One GmbH, Kremsmünster, Austria) with an 18-gauge needle to examine cortisol concentration. Pregnancy was confirmed by transrectal palpation. Swelling and edema of the vulva, the relaxation of the broad pelvic ligaments, the enlargement of the udder and the filling of the rear teats were evaluated on a 4-point scale according to Streyl et al. (2011). After this initial examination, enrolled heifers in the precalving pen were examined once per day to evaluate relaxation of the pelvic ligaments and filling of the rear teats. The aim of this examination was to exclude the possibility of calving in the next 24h as described in a previous study (Streyl et al., 2011). Approximately 5 to 3 d before calving, heifers were brought into the calving pen. In the calving pen, evaluation of the pelvic ligaments and examination of the rear teats were conducted twice daily at 0800 h and 2000 h. Heifers in the calving pen were observed hourly via direct observation of 1 trained investigator. Additionally, video surveillance was conducted 24 h/d. The video recordings were used to determine the time of the appearance of the calves' feet, if this event could not be observed directly.

The study protocol to evaluate the influence of DNH (Sensiblex) on calving ease started with the first appearance of the fetal claws outside the vulva, which was considered as the beginning of stage two of labor. Thirty minutes later 400 mg of DNH (i.e., 10 ml of Sensiblex) or Placebo (**PLA**; i.e., 10 ml of 0.9 % NaCl- B. Braun, Melsungen, Germany) was administered to the heifer subcutaneously. Both substances were clear and had a similar viscosity. Assignment to the 2 treatment groups was randomized utilizing a randomization list generated with Microsoft excel (version 2013; Microsoft Corp., Redmond, WA) before the study and blinded to the investigator. The time interval of 30 min after the onset of stage two of calving was chosen according to the specific product characterization. If the heifer calved spontaneously within 30 min after application (i.e., 60 min after onset of stage two), calving was categorized as unassisted. Calving was defined as the passage of the calf in anterior presentation and dorsal position through the vulva until both tarsal joints were visible outside the dam. If both forelegs of the calf and the head with both eyes were visible outside the vulva 30 min after treatment, the heifer was allowed to continue calving without intervention and was categorized as unassisted as well. If the calf was not born 30 min after treatment, an extraction of the calf was conducted. First, the heifer's anogenital region was cleaned with warm water and soap and a vaginal examination was carried out to rule out any malposition or inappropriate size of the calf. In both cases the heifer was excluded from the study. Without exception, all extractions were conducted in a lying position of the dam. If the heifer was standing the animal was cast first. Two female veterinarians and one member of the farm staff were involved in the pulling process. One veterinarian was responsible for the heifer, another veterinarian operated the force gauge, and 1 farm employee used the mechanical calf puller at the veterinarian's instructions. As soon as the heifer was lying in a sternal position, a mechanical calf puller was positioned behind the heifer. One calving rope with two loops on each end was attached to the calf's feet. A digital force gauge (PCE-FB2 k, PCE Deutschland GmbH, Meschede, Germany; Figure 1) was interposed between the ends of the calving rope and the mechanical calf puller using 2 carabiners. The calf puller was used with a constant tension; actual pulling was performed only when the heifer strained. The force gauge used for this study measured the pulling force 2 times per second starting with the first pull on the ropes with an accuracy of ± 0.1 % (up to 2,000 N). Results were continuously recorded on a secure digital memory card. Measurement ended when the calf was completely delivered. On average 3.0 ± 4.4 min after calving (i.e., unassisted or assisted calving), a blood sample was taken from the heifer's coccygeal vein using a Vacutainer system (Vacuette 8 mL Serum Beads Clot Activator, Greiner Bio-One GmbH, Kremsmünster, Austria). Blood samples were centrifuged at 3,000 x *g* for 10 min and serum was stored at -22 °C until analysis for cortisol concentration. Furthermore, after an assisted calving, a vaginal examination was carried out to identify calving trauma. An obstetrical examination was performed when the newborn calf was small to exclude the possibility of twins. Finally, the calf was tube fed with 3 l of colostrum. Dam and calf were separated and brought to a postpartum pen and a calf box, respectively.

Personal perception of calving ease was rated by 2 veterinarians on a 4-point scale (i.e., very easy, easy, difficult, very difficult). Serum samples were analyzed for cortisol concentration at a commercial laboratory (Synlab Laboratories, Berlin, Germany; accreditation number: D-PL-14016-01-00; accreditation body: DAkkS, Berlin, Germany) using an immunoassay based on chemiluminescence. We aimed to exclude risk factors that could confound the calving process. Abnormal presentation, position or posture lead to necessary corrective action. Due to animal welfare considerations integrity and vitality of calf and dam were our priority. If the time protocol was not met, the calving was excluded. We modified the classification of Wehrend et al. (2003) such that light pulling force ranged from 0 to 500 N, moderately heavy force from 501 to 1,000 N and heavy pulling force from 1,001 N to include all measured results.

Statistical Analysis

Data were entered into Excel spreadsheets (version 2013; Microsoft Corp.) and analyzed using SPSS for Windows (version 22.0; SPSS Inc., Munich, Germany). The area under the curve **(AUC)** enables statistical comparison between groups without comparing every single value separately (Kovács et al., 2014). The intensity and the changes over time can be described through the area under the curve (Fekedulegn et al., 2007). For pulling force over time the AUC was calculated with the pulling force displayed on the x-axis and the measured time in sec on the y-axis. Normality of distributions of continuous parameters (i.e., force x time, cortisol concentration, and duration of calving) was assessed by plotting and visually examining the data, calculating a quantile-quantile plot, and using the Shapiro-Wilk test. Equality of variances was determined using Levene's test. The association between duration of calving considering both unassisted and assisted calving (n = 83) and the difference in cortisol concentration before and after parturition was determined utilizing Pearson's correlation coefficient.

Generalized linear mixed models were used to determine the effect of treatment group, day of gestation, and birth weight of calves on force x time, the duration of the pulling, the duration of calving, and the concentration of cortisol in the heifer, respectively. All models were built in a backward stepwise manner according to the model building strategies provided by Dohoo et al. (2009). Heifer was considered as a random effect. Interactions were tested for all relevant parameters. The covariate structure was chosen based on the lowest Akaike information criterion value. Post hoc comparison was carried out applying LSD test. Treatment group was considered as the factor of interest for all models and was forced to remain in the model regardless of level of significance until all nonsignificant parameters were removed.

A binary logistic regression model was built to determine the effect of treatment on the calving modality (i.e., unassisted or assisted calving). The model was built again in a manual backward stepwise manner and according to the model building strategies provided by Dohoo et al. (2009). Odds ratios and confidence intervals were calculated for all significant parameters. Again, treatment was forced to remain in the model.

Finally, the influence of treatment group on the evaluation of calving through both obstetricians was calculated with the chi-squared test. For all tests, differences with $P < 0.05$ were considered statistically significant.

3.1.4 **Results**

Initially, 120 heifers were enrolled in the study. Data from 83 calvings (38 female calves, 45 male calves) were available for final analyses. Denaverine hydrochloride and PLA were administered to 41 and 42 heifers, respectively. Thirty-six heifers calved without assistance, and 47 heifers required calving assistance accoring to the study protocol. A total of 37 heifers (30.8 %) had to be excluded due to abnormal presentation, position, or posture of the calves (i.e., posterior presentation, lateral or ventral position, n = 6), oversized calves (i.e., cesarean section, n = 2), and failure to implement the underlying timeline (i.e., calving before administering of DNH or PLA, earlier intervention necessary because of a premature expulsion of the placenta, n = 29). In 3 calvings (7 %) data of pulling force could not be obtained due to technical failure of the force gauge. Hence, they were excluded from the analysis of pulling force. The duration of calving was measured and included in the analysis. Mean birth weight of calves was 41 ± 4.6 kg (range: 28-52 kg).

A sample size calculation was performed before initiation of the study (G*Power program, version 3.1.5; University of Düsseldorf, Düsseldorf, Germany). A difference of AUC (time x force) between both treatment groups was found in a pilot trial. Using $\alpha = 0.05$, 1-

β = 0.8, and a 2-tailed study design, a sample size of 51 heifers per treatment group was necessary to verify our hypothesis.

Twenty out of 41 heifers treated with DNH (49 %) and 16 out of 42 heifers treated with PLA (38 %) calved without assistance. There was no effect of treatment on calving modality (*P* = 0.524). The duration of pregnancy had a Picant influence on calving assistance (*P* = 0.016, odds ratio: 0.846; 95% CI: 0.738 – 0.970).

The average duration of unassisted calving in the DNH and PLA group was 57 min 48 s ± 13 min 36 s (n = 20) and 49 min 48 s ± 16 min 30 s (n = 16), respectively (*P* = 0.119). The average duration for all calvings (i.e., assisted and unassisted) was 66 min 42 s ± 14 min 54 s (n = 41) and 66 min 48 s ± 17 min 36 s (n = 42) for DNH and PLA treated heifers, respectively (*P* = 0.959). Calving assistance tended to be shorter in heifers treated with DNH (3 min 11 s ± 1 min 47 s) compared with PLA (3 min 59 s ± 3 min 5 s; *P* = 0.075).The maximum pulling force measured was 1,979.5 N. Duration of pulling ranged from 1 min to 12 min. Average duration was 3.64 ± 2.61 min.

The AUC was 64,373 ± 50,514 Ns (i.e., N x s) in DNH group and 91,553 ± 61,886 Ns in PLA group (*P* = 0.035) (Figure 2). The AUC comprises time x pulling force, which means both parameters are converted into one dimension to facilitate comparability. Figure 3 illustrates an example of pulling force measurement during calving assistance of 1 heifer.

A total of 166 cortisol samples (i.e., 1 sample before and 1 sample after calving of every heifer) were analyzed. The difference of cortisol concentration before and after parturition ranged from -4.61 to 210.34 ± 6.86 nmol/l in the DNH group and 0.8 to 174.92 ± 6.37 nmol/l in the PLA group. Mean difference in cortisol concentration before and after parturition regardless of treatment group was 105.8 ± 43.45 nmol/l. No effect of treatment was found on the increase of blood cortisol after parturition (*P* = 0.257). Cortisol differences were not correlated with duration of calving (r = 0.025; *P* = 0.819) or force x time (r = 0.059; *P* = 0.703). In addition, there was no influence of the treatment group on calving ease score (*P* = 0.284).

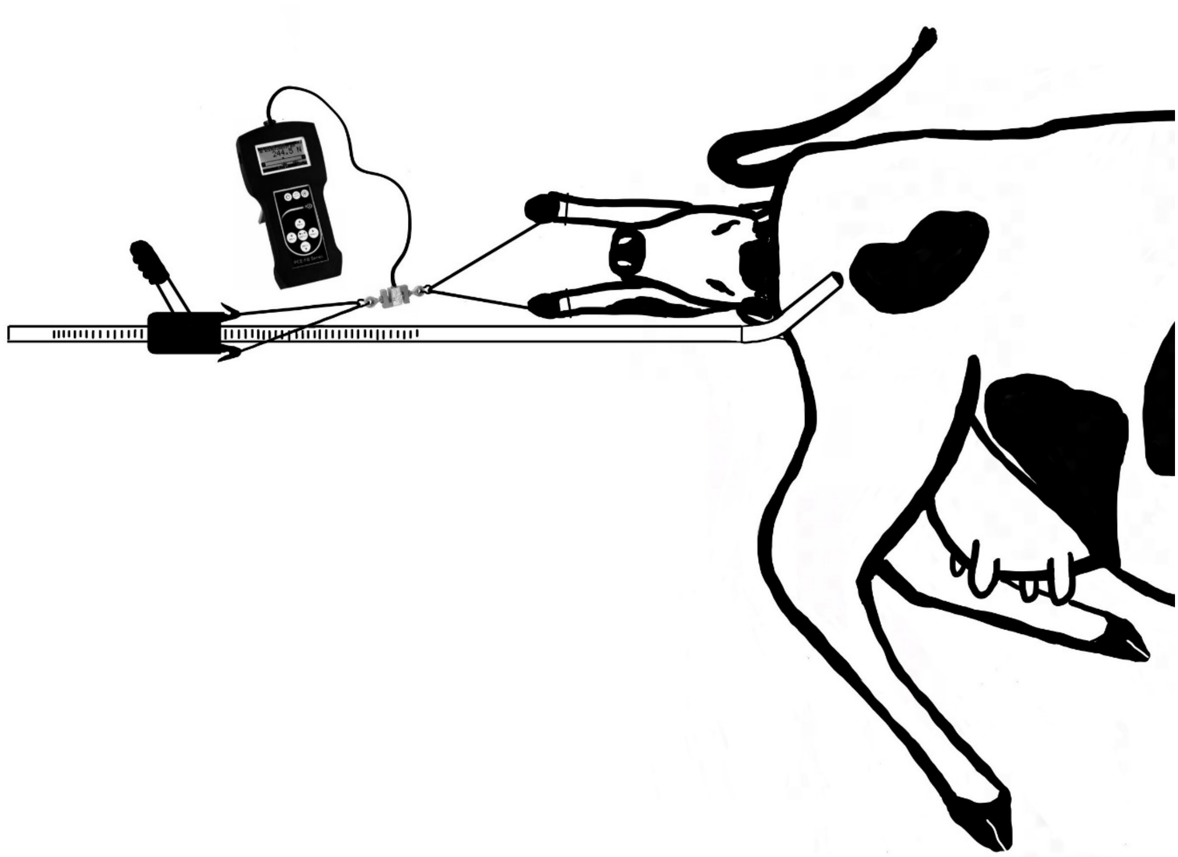


Figure 1. Use of calf puller and force gauge interposed between the calf's feet and the mechanical calf puller during calving assistance.

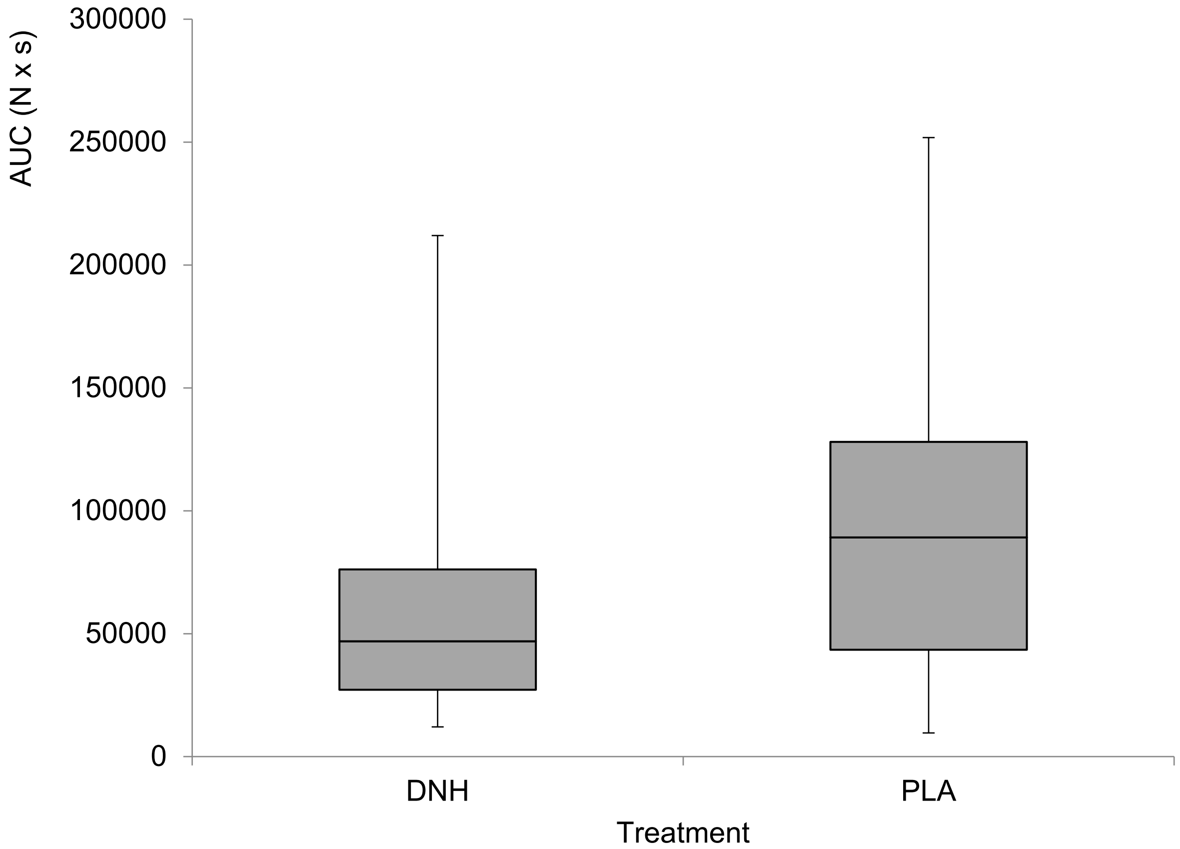


Figure 2. Boxplots of area under the curve (AUC; N x s) in the denaverine hydrochloride (DNH) and placebo (PLA) groups. The central box represents the interquartile range from the first to third quartiles. A segment inside the box shows the highest and lowest case within 1.5 times the interquartile range.

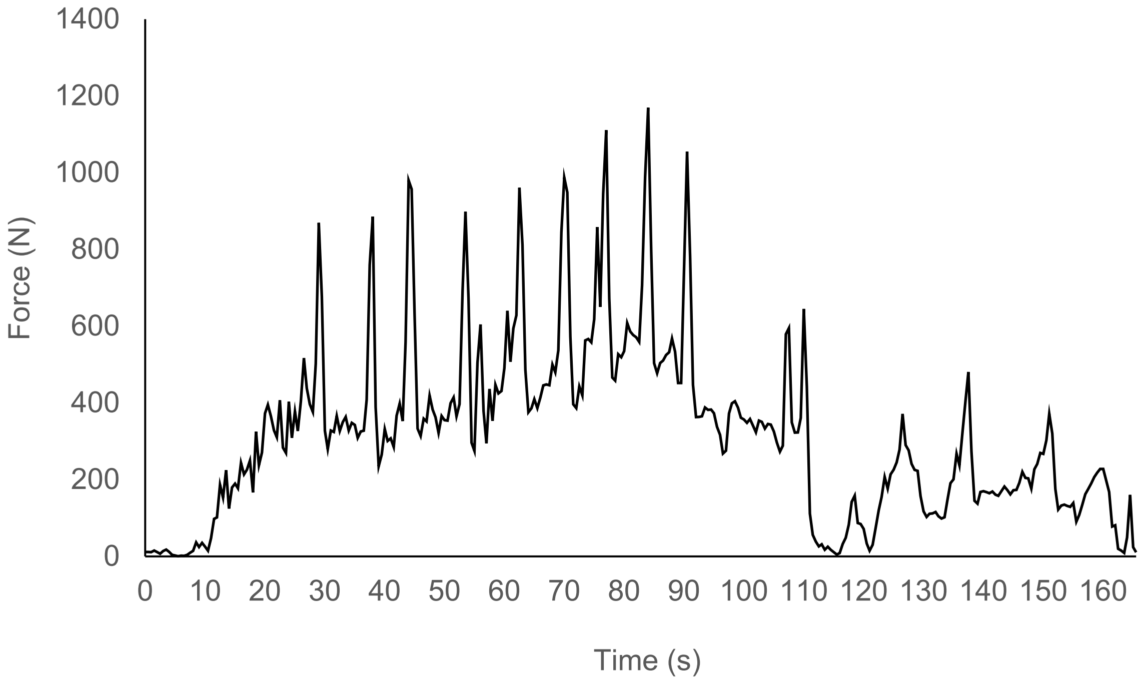


Figure 3. An example of force measurement during calving assistance of a heifer.

3.1.5 **Discussion**

In this study the effect of DNH on calving ease in heifers was tested. A drug with the label claim of easing calving is promising but also requires caution because it might lead to a reduced perception of the importance of a timely and correct calving assistance. Therefore, proving efficacy is of paramount importance. Measuring calving ease, however, was challenging because the process of calving is dependent on many factors (i.e., calf birth weight, maternal age and pelvis size, calving pen) and difficult to standardize. Because we assumed that duration of stage 2 could influence the calving progress and thus modify the effect of a given pulling force during assistance, we set out to keep the time of treatment administration in our study relative to the beginning of stage 2 constant (i.e., 30 min after onset of stage 2). Therefore, onset of calving was monitored by direct observation and 24-h video surveillance. A total of 83 heifers could be included in the analysis. A considerable number of heifers had to be excluded because it was impossible to meet the study protocol. Ten heifers calved in less than 30 min after the appearance of the calves' feet. In 19 heifers, we had to intervene before the 60 min limit was reached because the calf was at risk as indicated by a considerably blue colored tongue and clear lingual edema or premature placental expulsion. This study took place on a commercial dairy farm. For reasons of standardization this might have been advantageous. Nevertheless, future studies should implement different farms to increase external validity.

We decided to inject DNH or PLA 30 min after onset of stage two and to allow the heifer a total of 60 min for giving birth unassisted. Schuenemann et al. (2011) measured a duration of 45.8 min for heifers from the appearance of the amniotic sac outside the vulva (i.e., indicative of stage 2 of calving) until birth of the calf in unassisted calvings. The authors recommend starting calving assistance 65 min after the appearance of the feet. A nonsystematic review reported that calving assistance < 60 min after the appearance of the fetal hooves increased the risk of using a calf puller, duration of assistance, dystocia, and downer cows and reduced perinatal vigor (Mee, 2008). More recently, a high prevalence of retained placenta (78.9 %) and vulvovaginal lacerations (80 %) occurred in dams that experienced premature assistance (Kovács et al., 2016). The authors defined premature assistance as assistance <65 min after the appearance of the fetal hooves in the vulva. Eventually, they started assistance between 22 and 64 min (34.6 ± 10.5 min) after the amniotic sac appeared. In 2 recently published papers, the influence of systematic early calving assistance on calf health and calf survival was investigated (Villettaz Robichaud et al., 2017a,b). The authors achieved better results regarding calf mortality with obstetrical assistance within 15 min after the appearance of a calf's feet compared with a cutoff of 60 min. According to the manufacturer, DNH needs 15 to

30 min after injection until a spasmolytic effect occurs (Veyx-Pharma GmbH, 2009). Intervening in the calving process 60 min after the calves' feet appeared seemed to be a reasonable compromise between giving time for drug-mediated dilation without risking the negative effects of a prolonged calving.

There was no difference between both treatment groups in necessity of calving assistance. Presumably, the 60 min cutoff prevented or limited an effect of treatment. This hypothesis probably needs to be tested in another experimental set up. Furthermore, it must be considered that treatment with DNH eases the calving process but is not supposed to prevent calving assistance completely.

Usually, the degree of calving assistance is categorized based on subjective estimates (Hindson, 1978; Tenhagen et al., 2007) and is assessed on 3- to 5-point scales depending on the number of people and the extent of pulling (Meyer et al., 2001; Villettaz Robichaud et al., 2017a). Measuring pulling force with a technical device is more objective. In an in vitro model to simulate the extraction of calves the maximum mean traction force ranged from 352 ± 98 N to 597 ± 150 N depending on the traction technique (Becker et al., 2010). In this model the calf was pulled through an isolated pelvis; thus, resistance due to the soft tissues of the birth canal was missing. Few studies have been published that systematically measured pulling force in the field. Wehrend et al. (2003) evaluated pulling force for assisted calvings conducted with a mechanical calf puller in 24 cows. Peak values of up to 1,471 N were measured. By applying the modified classification, 20% of the calvings were assisted using heavy pulling force in the study of Wehrend et al. (2003). This is contrary to our study, where only light and moderate force was applied. This different distribution in pulling force most likely was caused because 95 % of the cows used by Wehrend et al. (2003) suffered from abnormal position, presentation and posture, which could have interrupted the calving process and resulted in higher pulling force. We excluded cases of abnormal position, presentation, and posture to achieve a more uniform population. Furthermore, time from the onset of calving until the onset of calving assistance varied widely (up to 4 h), which indicates that the calving process was probably pathologically prolonged.

The AUC for pulling force was smaller in the treatment group. This could indicate an effect of DNH such that the birth canal was softer and wider thus easing the calving process. Additionally, we suppose that the intensity of the pulling process (i.e., the duration of friction within the birth canal) negatively affects ease of calving because friction causes injury and inflammation in the tissue. This is in line with a previous study in which DNH and carbetocin increased the number of animals with a birth canal dilated > 25 cm and decreased time from the first examination to delivery as well as the need for assistance (Zobel and Taponen, 2014).

Additionally, fewer of birth canal lesions were found in the treatment group (Zobel and Taponen, 2014).

To quantify pain mediated stress during parturition (Nagel et al., 2016b), we measured the concentration of cortisol. The difference in cortisol concentration before and after parturition for every heifer was calculated, as the difference of cortisol concentration before and after a stressful event can indicate the stress level in cows (Bertulat et al., 2013). Blood cortisol concentrations differed before and after parturition in both treatment groups. A difference, however, was not found between the treatment and the control group. Civelek et al. (2008) described higher plasma cortisol concentrations in heifers with dystocia compared to heifers with eutocia. An increase in cortisol concentration after parturition was reported (Hydbring et al., 1999; Nagel et al., 2016b). The authors found a short-term increase in cortisol concentration which was considered as an acute stress response (Nagel et al., 2016b). We assume that this physiological activation of the hypothalamic-pituitary-adrenal axis, either as part of the endocrine pathway or as an acute stress response, was stronger and superimposed an effect of DNH treatment. Also, it is possible that the production of cortisol is not directly related to pulling force.

Further research is warranted to evaluate the influence of DNH on duration of the calving process and other stress and pain parameters such as heart rate variability or substance P (Coetzee 2011). Nevertheless, we conclude that DNH can reduce pulling force under field conditions if calving assistance is conducted but did not influence the cortisol response of the dam. Hence, careful and competent calving assistance is indispensable.

3.1.6 **Acknowledgements**

We greatly appreciate the support of Veyx-Pharma (Schwarzenborn, Germany) during the study. Furthermore, we gratefully thank the farm personnel of the dairy farm for the kind cooperation and for their patience and confidence demonstrated. Special thanks go to the staff of the Clinic for Animal Reproduction, Freie Universität Berlin, Germany, for their support during the practical part. Katrin Lange was partly funded by Tiergyn e.V. (Berlin, Germany).

3.1.7 **References**

Barrier, A. C. and M. J. Haskell. 2011. Calving difficulty in dairy cows has a longer effect on saleable milk yield than on estimated milk production. J. Dairy Sci. 94:1804-1812.

Barrier, A. C., M. J. Haskell, S. Birch, A. Bagnall, D. J. Bell, J. Dickinson, A. I. Macrae, and C. M. Dwyer. 2013. The impact of dystocia on dairy calf health, welfare, performance and survival. Vet. J. 195:86-90.

Becker, M., G. Tsousis, M. Lupke, F. Goblet, C. Heun, H. Seifert, and H. Bollwein. 2010. Extraction forces in bovine obstetrics: An in vitro study investigating alternate and simultaneous traction modes. Theriogenology 73:1044-1050.

Bertulat, S., C. Fischer-Tenhagen, V. Suthar, E. Mostl, N. Isaka, and W. Heuwieser. 2013. Measurement of fecal glucocorticoid metabolites and evaluation of udder characteristics to estimate stress after sudden dry-off in dairy cows with different milk yields. J. Dairy Sci. 96:3774‑3787.

BMJV, 2017. Tierschutzgesetz in der Fassung der Bekanntmachung vom 18. Mai 2006 (BGBl. I S. 1206, 1313), das zuletzt durch Artikel 141 des Gesetzes vom 29. März 2017 (BGBl. I S. 626) geändert worden ist.

Cardoso, C. S., M. J. Hotzel, D. M. Weary, J. A. Robbins, and M. A. von Keyserlingk. 2016. Imagining the ideal dairy farm. J. Dairy Sci. 99:1663-1671.

Caroprese, M., M. Albenzio, A. Marzano, L. Schena, G. Annicchiarico, and A. Sevi. 2010. Relationship between cortisol response to stress and behavior, immune profile, and production performance of dairy ewes. J. Dairy Sci. 93:2395-2403.

Civelek, T., H. A. Celik, G. Avci, and C. C. Cingi. 2008. Effects of Dystocia on Plasma Cortisol and Cholesterol Levels in Holstein Heifers and Their Newborn Calves. B Vet I Pulawy 52:649-654.

Coetzee, J. F. 2011. A review of pain assessment techniques and pharmacological approaches to pain relief after bovine castration: Practical implications for cattle production within the United States. Appl. Anim. Behav. Sci. 135:192‑213.

Coetzee, J. F., B. V. Lubbers, S. E. Toerber, R. Gehring, D. U. Thomson, B. J. White, and M. D. Apley. 2008. Plasma concentrations of substance P and cortisol in beef calves after castration or simulated castration. Am. J. Vet. Res. 69:751–762.

Dematawena, C. M. B. and P. J. Berger. 1997. Effect of Dystocia on Yield, Fertility, and Cow Losses and an Economic Evaluation of Dystocia Scores for Holsteins. J. Dairy Sci. 80:754-761.

Dockweiler, J. C., J. F. Coetzee, L. N. Edwards-Callaway, N. M. Bello, H. D. Glynn, K. A. Allen, M. E. Theurer, M. L. Jones, K. A. Miller, and L. Bergamasco. 2013. Effect of castration method on neurohormonal and electroencephalographic stress indicators in Holstein calves of different ages. J. Dairy Sci. 96:4340-4354.

Dohoo, I. R., S. W. Martin, and H. Stryhn. 2009. Veterinary Epidemiologic Research. University of Prince Edward Island, Charlottetown, PEI, Canada.

Dubuc, J., T. F. Duffield, K. E. Leslie, J. S. Walton, and S. J. LeBlanc. 2010. Risk factors for postpartum uterine diseases in dairy cows. J. Dairy Sci. 93:5764-5771.

Dyer, R. M., N. K. Neerchal, U. Tasch, Y. Wu, P. Dyer, and P. G. Rajkondawar. 2007. Objective Determination of Claw Pain and Its Relationship to Limb Locomotion Score in Dairy Cattle. J. Dairy Sci. 90:4592-4602.

Farm Animal Welfare Council. 1993. Second Report on Priorities for Research and Development in Farm Animal Welfare. Ministry of Agriculture, Fisheries and Food, London, UK.

Fekedulegn, D. B., M. E. Andrew, C. M. Burchfiel, J. M. Violanti, T. A. Hartley, L. E. Charles, and D. B. Miller. 2007. Area under the curve and other summary indicators of repeated waking cortisol measurements. Psychosom. Med. 69:651-659.

Funnell, B. J. and W. M. Hilton. 2016. Management and Prevention of Dystocia. Vet. Clin. North Am. Food Anim. Pract. 32:511-522.

Gleerup, K. B., P. H. Andersen, L. Munksgaard, and B. Forkman. 2015. Pain evaluation in dairy cattle. Appl. Anim. Behav. Sci. 171:25-32.

Heinrich, A., T. F. Duffield, K. D. Lissemore, and S. T. Millman. 2010. The effect of meloxicam on behavior and pain sensitivity of dairy calves following cautery dehorning with a local anesthetic. J. Dairy Sci. 93:2450-2457.

Hindson, J. C. 1978. Quantification of obstetric traction. Vet. Rec. 102:327-332.

Huber, J., T. Arnholdt, E. Möstl, C. C. Gelfert, and M. Drillich. 2013. Pain management with flunixin meglumine at dehorning of calves. J. Dairy Sci. 96:132-140.

Hüller, H. 1970. Pharmakologie und Klinik von O-(2-Athylbutoxy)-benzilsaure-2-dimethylaminoäthylesterhydrochlorid (= Spasmalgan). Zentralbl. Pharm. 109.

Huxley, J. N. and H. R. Whay. 2006. Current attitudes of cattle practitioners to pain and the use of analgesics in cattle. Vet. Rec. 159:662-668.

Huzzey, J. M., D. V. Nydam, R. J. Grant, and T. R. Overton. 2011. Associations of prepartum plasma cortisol, haptoglobin, fecal cortisol metabolites, and nonesterified fatty acids with postpartum health status in Holstein dairy cows. J. Dairy Sci. 94:5878‑5889.

Hydbring, E., A. Madej, E. MacDonald, G. Drugge-Boholm, B. Berglund, and K. Olsson. 1999. Hormonal changes during parturition in heifers and goats are related to the phases and severity of labour. J. Endocrinol. 160:75-85.

Kovács, L., F. L. Kézér, and O. Szenci. 2016. Effect of calving process on the outcomes of delivery and postpartum health of dairy cows with unassisted and assisted calvings. J. Dairy Sci. 99:7568-7573.

Kovács, L., J. Tőzsér, O. Szenci, P. Póti, F. L. Kézér, F. Ruff, G. Gábriel-Tőzsér, D. Hoffmann, M. Bakony, and V. Jurkovich. 2014. Cardiac responses to palpation per rectum in lactating and nonlactating dairy cows. J. Dairy Sci. 97:6955-6963.

Lombard, J. E., F. B. Garry, S. M. Tomlinson, and L. P. Garber. 2007. Impacts of dystocia on health and survival of dairy calves. J. Dairy Sci. 90:1751-1760.

Mainau, E. and X. Manteca. 2011. Pain and discomfort caused by parturition in cows and sows. Appl. Anim. Behav. Sci. 35:241-251.

Mee, J. F. 2004. Managing the dairy cow at calving time. North Am. Food Anim. Pract. 20:521-546.

Mee, J. F. 2008. Prevalence and risk factors for dystocia in dairy cattle: a review. Vet. J. 176:93-101.

Meyer, C. L., P. J. Berger, K. J. Koehler, J. R. Thompson, and C. G. Sattler. 2001. Phenotypic trends in incidence of stillbirth for Holsteins in the United States. J. Dairy Sci. 84:515-523.

Mormède, P., S. Andanson, B. Aupérin, B. Beerda, D. Guémené, J. Malmkvist, X. Manteca, G. Manteuffel, P. Prunet, C. G. van Reenen, S. Richard, and I. Veissier. 2007. Exploration of the hypothalamic–pituitary–adrenal function as a tool to evaluate animal welfare. Physiol. Behav. 92:317-339.

Möstl, E., and R. Palme. 2002. Hormones as indicators of stress. Domest. Anim. Endocrinol. 23:67‑74.

Nagel, C., J. Aurich, L. Trenk, N. Ille, M. Drillich, W. Pohl, and C. Aurich. 2016a. Stress response and cardiac activity of term and preterm calves in the perinatal period. Theriogenology 86:1498-1505.

Nagel, C., L. Trenk, C. Aurich, N. Ille, M. Pichler, M. Drillich, W. Pohl, and J. Aurich. 2016b. Sympathoadrenal balance and physiological stress response in cattle at spontaneous and PGF(2 alpha)-induced calving. Theriogenology 85:979-985.

Negrao, J. A., M. A. Porcionato, A. M. de Passille, and J. Rushen. 2004. Cortisol in saliva and plasma of cattle after ACTH administration and milking. J. Dairy Sci. 87:1713-1718.

Noakes, D. E., T. J. Parkinson, and G. C. W. England. 2001. Parturition and the care of parturient animals. Pages 155-187 in Arthur's Veterinary Reproduction and Obstetrics (Eighth Edition). W.B. Saunders, Oxford.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Saco, Y., M. Fina, M. Giménez, R. Pato, J. Piedrafita, and A. Bassols. 2008. Evaluation of serum cortisol, metabolic parameters, acute phase proteins and faecal corticosterone as indicators of stress in cows. Vet. J. 177:439-441.

Schuenemann, G. M., I. Nieto, S. Bas, K. N. Galvao, and J. Workman. 2011. Assessment of calving progress and reference times for obstetric intervention during dystocia in Holstein dairy cows. J. Dairy Sci. 94:5494-5501.

Streyl, D., C. Sauter-Louis, A. Braunert, D. Lange, F. Weber, and H. Zerbe. 2011. Establishment of a standard operating procedure for predicting the time of calving in cattle. J. Vet. Sci. 12:177-185.

Tenhagen, B. A., A. Helmbold, and W. Heuwieser. 2007. Effect of various degrees of dystocia in dairy cattle on calf viability, milk production, fertility and culling. J Vet Med A Physiol Pathol Clin Med 54:98-102.

USDA. 2017. Animal Welfare Act and Animal Welfare Regulations. Accessed Mar. 28, 2018. https: / / www .aphis .usda .gov/animal welfare/ downloads/ AC BlueBook AWA FINAL 2017_508comp.pdf.

Veyx-Pharma GmbH, S. 2009. Fachinformation Sensiblex 40 mg/ml. (Zulassungsnummer 3100278.00.00). Veyx-Pharma Gmb, H. 2009. Fachinformation Sensiblex 40 mg/mL. (Zulassungsnummer 3100278.00.00.) Veyx-Pharma GmbH, Schwarzenborn, Germany.

Villettaz Robichaud, M. V., D. L. Pearl, S. M. Godden, S. J. LeBlanc, and D. B. Haley. 2017a. Systematic early obstetrical assistance at calving: I. Effects on dairy calf stillbirth, vigor, and passive immunity transfer. J. Dairy Sci. 100:691‑702.

Villettaz Robichaud, M. V., D. L. Pearl, S. M. Godden, J. Rushen, S. J. LeBlanc, and D. B. Haley. 2017b. Systematic early obstetrical assistance at calving: II. Effects on dairy heifer calf growth, health, and survival to weaning. J. Dairy Sci. 100:703‑712.

von Borell, E., J. Langbein, G. Despres, S. Hansen, C. Leterrier, J. Marchant-Forde, R. Marchant-Forde, M. Minero, E. Mohr, A. Prunier, D. Valance, and I. Veissier. 2007. Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals - A review. Physiol. Behav. 92:293-316.

Wehrend, A., K. B. Padberg, and H. Bostedt. 2003. [Electronic quantification of traction force during use of a mechanical calving aid in bovine parturition]. Berl. Munch. Tierarztl. Wochenschr. 116:12-19.

Whitlock, B. K., E. A. Coffman, J. F. Coetzee, and J. A. Daniel. 2012. Electroejaculation increased vocalization and plasma concentrations of cortisol and progesterone, but not substance P, in beef bulls. Theriogenology 78:737-746.

Zobel, R. and J. Taponen. 2014. Denaverine hydrochloride and carbetocin increased welfare during and after parturition and enhanced subsequent fertility in cattle. J Dairy Sci. 97:3570-3577.

3 DISCUSSION

Calving is the crucial moment in the life cycle of a dairy cow (Kovács et al., 2016). Optimizing the calving process could be the driver for better health, productivity and persistence. The overall objective of our thesis was to predict stage 2 of calving and to examine the impact of DNH on calving ease in heifers and calves. An accurate prediction of the calving time is critical for reasons of animal welfare and efficiency (Saint-Dizier and Chastant-Maillard, 2015). Although calving is a daily routine on dairy farms, prediction of calving using technical devices (e.g., inclinometers and accelerometers, vaginal sensors) lacks scientific support regarding different types of cattle breeds and different calving conditions (Saint-Dizier and Chastant-Maillard, 2015). Various prediction methods based on changes in precalving behavior exist. Hence, several recommendations regarding quantity of observations or relevant parameter for prediction of calving can be found in literature (Mee 2004; Saint-Dizier and Chastant-Maillard, 2015; Shah et al., 2006; Streyl et al., 2011).

Easing the calving process is the other important objective of this thesis. The influence of DNH on calving ease, calving duration and stress parameters in the dam and calf was studied. This required determining the beginning of calving as accurately as possible.

The aim of the first study was to predict calving via direct observation and evaluation of teat filling and relaxation of the pelvic ligaments. Changes in behavior and physical alteration before calving have frequently been reported (Miedema et al., 2011b; Jensen 2012; Proudfoot et al., 2013). Except for one sign of imminent parturition, the interobserver reliability was good to very good. This was the first prerequisite for a sound calving prediction via direct observation. Tail raising, clear vaginal discharge and bloody vaginal discharge were more likely to occur 24h before calving than in late pregnancy, which supports the importance of these signs. Nevertheless, the results of this study demonstrated that alteration in behavior and clinical signs pre-calving are animal specific. A pattern in the occurrence of SIP was not found. This restricts its applicability and necessitates further research.

An increase in turning the head toward the abdomen during the last two hours before calving has been described (Jansen 2012). We could not find such a behavioral increase nor did a significant difference exist between the control and the calving period. This discrepancy might result from the different observation methods implemented in both studies. Continuous observation enables recording every SIP during the observation period whereas repetitive observations with a proportionally short duration (15 s) bears the risk of missing certain SIP. To record the complete behavioral repertoire for the time before parturition continuously monitoring as implemented in several other studies (e.g. Miedema et al., 2011a; Barrier et al., 2012; Jensen, 2012) is inevitable. Nevertheless, our study was conducted with the scope of field conditions to simulate common calving monitoring practices. Hence the implemented regular, repetitive short observations appeared reasonable and in line with current best practice.

We created an equation based on the SIP data to predict hours until parturition. As soon as an expanded database of SIP ensures more reliable results, this prediction model might be a promising attempt to provide practical recommendations. In the future such an equation might be included in an application tool to ease on farm calving prediction. In a recently published paper, a prediction model based on data of rumination behavior was presented (Zehner et al., 2018). Due to insufficient performance the model itself was not suitable for practical application. Still there seems to be an increasing interest in the idea of a prediction models based on physiological data before calving.

Streyl et al. (2011) evaluated teat filling and relaxation of the pelvic ligaments using a score to predict no calving within 12 h in 95.5 % of the heifers. Our results were comparable enabling exclusion of calving in the next 12 h. Results were ranked according to the day of gestation. A cut-off value of > 5 points combining pelvic ligaments (**PL**) double weighted and teat filling (**TF**) resulted in a negative predictive value of 96.8 whereas sensitivity was 69.0 and specificity 87.9. In approximately 97 % did heifers not calve during the next 12 h when they were diagnosed as not calving according to the parturition score. Results for predicting calving based on both parameters were not satisfying. Excluding the possibility of calving facilitates calving monitoring and improves the decision when to move heifers to a maternity pen. However, the evaluation of relaxation of the pelvic ligaments and teat filling is not accurate enough for calving prediction.

Another important aspect of this thesis was the evaluation of calving ease in dams and calves after treatment with DNH in a controlled, randomized and blinded trial. The calving process itself means pain and effort to the dam. Especially primiparous dams suffer a lot from dystocia (Lombard et al., 2007). Reliable calving prediction and reasonable calving management are key factors for healthy dams and calves (Saint-Dizier et al., 2015; Villettaz Robichaud et al., 2017). Therefore, medical treatment during parturition might ease the calving process and optimize animal welfare. Surprisingly, data of clinical studies evaluating the effect of DNH during calving is limited. Considering own experiences resulting from the second study, we assume that the challenge of standardizing the process of calving might be one important reason. To enable comparability, a consistent and strict time line was pursued. When the time line was not met, the calving procedure was excluded from analysis. This differs from the experimental set up by Wehrend et al. (2003), where pulling force was measured after farm staff unavailingly tried to develop the calf. We decided to allow the heifers 60 min time to calve (i.e., from the appearance of at least one fetal hoof to the passage in anterior position through the vulva) with injection of DNH or placebo (**PLA**) 30 min after the beginning of stage 2 of calving. The time of intervention (i.e., onset of calving assistance) is certainly a debatable issue. Traditionally a rule of thumb recommended to wait for two hours (starting as soon as both feet are visible) until onset of calving assistance (Mee 2004). A more recent study suggested a considerably shorter time span of only 65 min from the appearance of one foot outside the vulva until the start of assistance (Schuenemann et al., 2011). The authors recommend this reference time based on their observation that calving duration in heifers was 45.8 min from the appearance of the amniotic sac outside the vulva until the calf was born without assistance. Moreover, other authors achieved better results regarding calf mortality conducting calving assistance 15 min after the appearance of a calf's foot compared to an intervention 60 min after the appearance, respectively (Villettaz Robichaud et al., 2017a,b). Hence, intervening in the calving process 60 min after the beginning of stage two appeared to be a reasonable compromise between giving time for drug-mediated dilation without risking disadvantages for dam and calf due to a prolonged calving.

To quantify stress during parturition, cortisol concentration in serum was measured before and after calving in both treatment groups. In both treatment groups a significant increase of cortisol concentration after calving was found, which is in line with previous studies (Hydbring et al., 1999; Nagel et al., 2016a). This reflects a calving induced stress response shown by maternal cortisol release (Nagel et al., 2016a). We could not find a difference between both treatment groups (P = 0.257). A possible explanation could be that the effect of DNH is superimposed by the stressful and painful calving event.

Area under the curve (**AUC**) of pulling force was smaller in the DNH group than in the PLA group, respectively. We suppose that the birth canal was softer and wider due to the agent and thus the calving process was eased. As we could not find a comparable double blinded field study, results are difficult to compare and interpret. In another study, in which a long acting oxytocin (carbetocin) and DNH were applied during calving assistance, an increased number of cows treated with both drugs had a more dilated birth canal, decreased time from first examination to delivery and a reduced need for assistance (Zobel and Taponen, 2014).

No significant difference between both treatment groups was found regarding duration of calving assisted and unassisted, respectively. A tendency was found that calving assistance was shorter in heifers treated with DNH compared to PLA group (P = 0.075). Further research is warranted to evaluate the influence of DNH on duration of the calving process.

The APGAR score was originally developed for human babies to evaluate their chance of survival (Apgar et al., 1958). The score was adjusted to veterinary medicine (Mülling 1977). It was intended to identify compromised calves but according to Homerosky et al. (2017) it has failed to be adopted outside research fields. However, there was no significant difference in Apgar score between both treatment groups.

Measuring of lactate concentration in blood is another method to identify compromised calves after parturition (Sorge et al., 2009). A difference between both treatment groups was not found. We assume that the on-farm stress measuring in newborn calves is challenging, as the transition from the uterine to the extra uterine environment is presumably more stressful than the alleviating effect of DNH during calving.

However, there is a measurable effect of DNH on pulling force during calving assistance. Further research concerning the influence of DNH on stress and pain related parameter in dam and calf is warranted.

Despite alleviating calving assistance by medical treatment, careful and qualified calving assistance is inevitable for a good outcome for heifer and calf.

4 SUMMARY

4.1 Calving prediction and evaluation of calving ease after medical treatment in Holstein-Friesian heifers

The overall objectives of this thesis were to predict calving via direct observation and to evaluate calving ease after medical treatment in Holstein Friesian heifers and their calves.

Calving is the crucial event in the life cycle of a dairy cow. Recognizing the beginning of calving, especially the beginning of stage 2 of calving, facilitates calving management and enables an optimal start for cow and calf. We aimed to predict calving dispensing with technical equipment. Prior to the study six signs of imminent parturition were identified based on a literature research and systematic assessment. We intended to develop a reliable prediction method with regard to the second study. Reliable calving prediction facilitates conducting a field study whereas the beginning of stage 2 of calving is important to recognize. Hence, the first study examined physical and behavioral alterations in heifers shortly before parturition. We intended to predict calving without technical equipment via direct observation and evaluation of relaxation of the pelvic ligaments and teat filling.

In a first experiment, the interobserver reliability of SIP was tested. Except for one case, cohen's kappa results were good or very good. During the second experiment SIP was tested under field condition. Thirty-seven heifers from day 267 ± 4 d of parturition were observed hourly for 15 sec each. If one SIP was seen, it was ticked off in a list. Additionally, the relaxation of the pelvic ligaments and teat filling was evaluated every 12 hours.

Compared to a precalving control period (day 4 for before calving) tail raising, clear vaginal discharge and bloody vaginal discharge were significantly more likely to occur 24 h before calving. Lying lateral with abdominal contractions was excluded because it was not observed during the control period. Additionally, an equation was built to predict the hours until parturition. In a first version the absence or presence of every SIP except turning the head toward the abdomen was included. In a second version, hours until parturition were estimated with the factors days of gestation, tail raising and clear vaginal discharge. As SIP are animal specific, they are no precise instrument for calving prediction. However, the equation is only a first approach to develop a formula, that might be included in a calving prediction application. Further research is necessary to identify pattern in occurrence of SIP and to ensure a reliable basis for calving prediction.

A cut-off value of > 5 points combining PL double weighted and TF resulted in a negative predictive value of 96.8 whereas sensitivity was 68.97 and specificity 87.91. Hence, the examination of PL and TF was not satisfying for predicting calving within the next 12 hours. Consistent with previous findings, results for excluding calving were considerably higher. Overall, the prediction of stage two of calving via direct observation and evaluation of PL and TF was not sufficient. Nevertheless, further research into the occurrence of SIP is warranted to enable a solid basis for an equation as a prediction tool.

The second study was conducted to examine the effects of DNH on calving ease in heifers and calves. A study protocol with a concise timeline was developed to enable comparable results. The beginning of stage two was defined as the first time one or both of the calves' feet became visible. Thirty minutes later, DNH or a Placebo (NaCl) was administered to the heifer subcutaneously. If calving was completed during the next 30 minutes, the case was recorded as unassisted. Duration of calving was measured and blood samples were drawn for cortisol and lactate concentration in heifers and calves, respectively. If calving was not completed 60 min after beginning of stage 2, calving assistance was conducted. A mechanical calf puller was utilized to ensure a comparable extraction method. Furthermore, a digital force gauge was attached to the calves' feet and the mechanical calf puller. Pulling force was measured during calving assistance. Finally, blood samples were drawn, heifers were examined for vaginal lacerations and calves were supplied with colostrum.

Data from 83 calvings were included in the final analyses. Nearly 50 % of heifers treated with DNH and 38 % of heifers treated with PLA calved without assistance ($P = 0.524$). Duration of calving in both groups did not differ significantly, but a tendency was found that calving assistance was shorter in heifers treated with DNH (3 min 11 s ± 1 min 47 s) compared to PLA (3 min 59 s ± 3 min 5 s; P = 0.075).

There was a significant difference of concentration of cortisol before and after calving in both treatment groups. A difference in the increase of cortisol concentration after calving between treatment groups was not found (P = 0.257). The authors speculate that the stressful event itself superimposed the effect of DNH during parturition.

The area under the curve was estimated to enable comparison of pulling force data. The AUC was significantly smaller in the DNH treatment group compared to PLA (P = 0.035). This result indicates the influence of DNH on the calving process. We assume that the widening and softening of the birth canal facilitates the delivery of the calf.

The vitality of the newborn calves was evaluated by appliance of the APGAR score. Moreover, l-lactate concentration in blood of the calves was measured directly after calving with a validated handheld meter. Both parameters were not influenced by the treatment group.

Overall, the first study clearly demonstrated that further research is warranted to achieve a reliable prediction method. There is a rapid and ongoing progress in the development of technical devices for calving prediction. Still, a better understanding of behavior and physical alterations before calving is essential.

A measurable effect of DNH on pulling force was found in the second study. We assume that this means an improvement in the calving process for the dam. For evaluation of stress in dam and calf, further research is warranted.

5 ZUSAMMENFASSUNG

5.1 Geburtsvorhersage und Beurteilung der Geburtserleichterung nach Behandlung von Holstein-Friesian Färsen

Die wichtigsten Ziele dieser Arbeit waren die Vorhersage der Kalbung sowie die Beurteilung der Geburtserleichterung nach Behandlung von Holstein-Friesian Färsen.

Die Kalbung ist der entscheidende Moment im Lebenszyklus einer Milchkuh. Das Erkennen der nahenden Geburt, besonders ihrer zweiten Phase, vereinfacht das Abkalbemanagement und ermöglicht einen optimalen Start für Mutter und Kalb. Wir beabsichtigten die Kalbung ohne den Einsatz von technischen Geräten vorherzusagen. Vor Beginn der Studie wurde eine Literaturrecherche durchgeführt, aus der sich schließlich sechs signs of imminent parturition (SIP) ergaben. Ziel war es, eine verlässliche Vorhersagemethode zu entwickeln, um auch für weitere Studien gute Voraussetzungen zu schaffen, bei denen der Beginn der zweiten Geburtsphase genau vorhergesagt werden muss. Folglich, wurden in der ersten Studie physische – sowie Verhaltensveränderungen bei Färsen kurz vor der Geburt untersucht. Ziel war eine Vorhersage ohne technische Ausstattung mithilfe von direkter Überwachung und Bewertung der Beckenbänder und der Zitzenfüllung.

In einem ersten Versuch wurde die Verlässlichkeit zwischen Beobachtern (interobserver reliability) untersucht. Abgesehen von einem Fall waren die Ergebnisse für cohen's kappa gut oder sehr gut. Während des zweiten Experiments wurden die SIP unter Feldbedingungen getestet. Insgesamt 37 Färsen vom 267 ± 4 d nach der Besamung wurden stündlich für je 15 s beobachtet. Wenn ein SIP auftauchte, wurde es in einer Liste vermerkt. Zusätzlich sind die Entspannung der Beckenbänder sowie die Zitzenfüllung alle 12 h untersucht worden.

Im Vergleich zu einem Kontrollzeitraum (4 d vor der Abkalbung), traten Schwanzabhalten, klarer Vaginalausfluss und blutiger Vaginalausfluss signifikant häufiger in den letzten 24 h vor der Kalbung auf. Seitenlage mit Bauchpresse wurde aus dieser Rechnung ausgeschlossen, da es im Kontrollzeitraum nicht auftrat. Zusätzlich wurde eine Formel entwickelt, um die Stunden bis zur Geburt vorherzusagen. In der ersten Variante waren alle SIP, bis auf Kopf zum Bauch drehen, miteingeschlossen. In der zweiten Variante wurden die Stunden bis zur Geburt mit den Faktoren Trächtigkeitstag, Schwanzabhalten und klarer Vaginalausfluss berechnet. Da SIP sehr individuell auftreten, sind sie kein genaues Vorhersageinstrument. Die Formel ist als erste Annäherung zu betrachten, die vielleicht zukünftig in eine Kalbungsvorhersage-App eingebaut werden könnte. Weitere

Untersuchungen sind notwendig, um ein Muster im Auftreten der SIP zu finden und eine solide Basis zur Geburtsvorhersage zu gewährleisten

Ein Schwellenwert von > 5 Punkten aus der Kombination Beckenbänder doppelt - und Zitzenfüllung einfach gewichtet führte zu einem negativen prädiktiven Wert von 96,8, mit einer Sensitivität von 69,0 und einer Spezifität von 87,9. Folglich war die Geburtsvorhersage anhand der Untersuchung von Beckenbändern und Zitzenfüllung nicht zufriedenstellend, um die Geburt in den nächsten 12 h vorherzusagen. Vergleichbar mit vorherigen Studienergebnissen waren die Ergebnisse deutlich besser, wenn die Kalbung in den nächsten 12 h ausgeschlossen wurde. Insgesamt war die Vorhersage anhand von SIP und Beckenbändern und Zitzenfüllung nicht ausreichend. Trotzdem ist weitere Forschung sinnvoll, damit SIP vielleicht doch als Formel in ein Vorhersagetool eingesetzt werden können.

Die zweite Studie wurde durchgeführt, um die Auswirkungen von Denaverinhydrochlorid (DNH) auf Geburtserleichterung zu testen. Ein Studienprotokoll mit einer klaren zeitlichen Abfolge wurde entwickelt, um vergleichbare Ergebnisse zu bekommen. Der Beginn der zweiten Geburtsphase wurde definiert als der Zeitpunkt, an dem zum ersten Mal mindestens eine Klaue des Fetus sichtbar wurde. Dreißig Minuten später wurde DNH oder Placebo (NaCl) unter die Haut der Färsen injiziert. Falls die Färse in den weiteren 30 Minuten gekalbt hatte, wurde die Geburt als spontan bewertet. Die Dauer der Geburt wurde gemessen und Blutproben wurden genommen, um Cortisol im Blut der Färsen und Lactat im Blut der Kälber zu bestimmen. Falls die Geburt nach insgesamt 60 min nach Beginn der zweiten Geburtsphase noch nicht abgeschlossen war, wurde Geburtshilfe geleistet. Dazu wurde ein mechanischer Geburtshelfer verwendet, um eine vergleichbare Auszugsmethode sicherzustellen. Ein digitales Kraftmessgerät wurde zwischen Geburtshelfer und den Vordergliedmaßen der Kälber befestigt. Zugkraft wurde während der Geburtshilfe gemessen. Schließlich wurden ebenfalls Blutproben genommen, die Färsen wurden geburtshilflich auf Verletzungen untersucht und die Kälber mit Kolostrum versorgt.

Die Daten von 83 Geburten konnten in die abschließende Auswertung aufgenommen werden. Ungefähr die Hälfte der mit DNH behandelten Färsen und 38 % der mit einem Placebo behandelten Färsen, kalbten ohne Geburtshilfe ($P = 0.524$). Die Dauer der Geburt unterschied sich nicht zwischen beiden Behandlungsgruppen. Die Dauer der Geburtshilfe in der DNH Gruppe war numerisch etwas kürzer (3 min 11 s ± 1 min 47 s) im Vergleich zur Placebogruppe (3 min 59 s ± 3 min 5 s; P = 0.075).

Beim Vergleich der Kortisolkonzentrationen vor und nach der Kalbung gab es in beiden Behandlungsgruppen jeweils signifikante Unterschiede. Keine signifikanten Unterschiede konnten hingegen im Anstieg der Kortisolkonzentration im Vergleich der beiden

Behandlungsgruppen miteinander festgestellt werden (P = 0,275). Möglicherweise hat die durch die Geburt entstandene Stresssituation die Wirkung von DNH überlagert.

Die Fläche unter der Kurve (errechnet aus den Parametern Geburtsdauer und Auszugskraft) war signifikant kleiner in der DNH Gruppe im Vergleich zur Placebogruppe (P = 0.035). Das könnte einen Einfluss von DNH auf den Geburtsvorgang zeigen. Wir nehmen an, dass die Geburtswegerweichung und -erweiterung durch DNH die Entwicklung des Kalbs vereinfacht.

Die Vitalität der neugeborenen Kälber wurde mithilfe des APGAR Schemas beurteilt. Außerdem wurde direkt nach der Geburt aus dem Blut der Kälber L-Laktat mittels eines validierten Handmessgerätes bestimmt. Die Behandlungsgruppe zeigte keinen Einfluss auf die Parameter.

Insgesamt zeigte die erste Studie, dass noch weitere Untersuchungen notwendig sind, um eine verlässliche Vorhersagemethode zu entwickeln. Außerdem gibt es einen stetigen technischen Fortschritt bezüglich der Entwicklung von technischen Geräten zur Geburtsvorhersage. Trotzdem ist ein ausgeprägteres Wissen um die physischen - sowie die Verhaltensveränderung vor der Kalbung notwendig.

Ein messbarer Effekt von DNH auf die Zugkraft wurde in der zweiten Studie gefunden. Wir nehmen an, dass das eine Verbesserung für das Muttertier darstellt. Für die Beurteilung der Stressparameter müssen noch weitere Untersuchungen durchgeführt werden.

6 REFERENCES FOR INTRODUCTION AND DISCUSSION

Apgar, V. 1953. A proposal for a new method of evaluation of the newborn infant. Curr. Res. Anesth. Analg. 32:260-267.

Apgar, V., D. A. Holaday, L. S. James, I. M. Weisbrot, and C. Berrien. 1958. Evaluation of the newborn infant; second report. J. Am. Med. Assoc. 168:1985-1988.

Barrier, A. C., M. J. Haskell, S. Birch, A. Bagnall, D. J. Bell, J. Dickinson, A. I. Macrae, and C. M. Dwyer. 2013. The impact of dystocia on dairy calf health, welfare, performance and survival. Vet. J. 195:86–90.

Barrier, A. C., E. Ruelle, M. J. Haskell, and C. M. Dwyer. 2012b. Effect of a difficult calving on the vigour of the calf, the onset of maternal behaviour, and some behavioural indicators of pain in the dam. Prev. Vet. Med. 103:248-256.

Becker, M., G. Tsousis, M. Lupke, F. Goblet, C. Heun, H. Seifert, and H. Bollwein. 2010. Extraction forces in bovine obstetrics: An in vitro study investigating alternate and simultaneous traction modes. Theriogenology 73:1044-1050.

Berglund, B., J. Philipsson, and O. Danell. 1987. External signs of preparation for calving and course of parturition in Swedish dairycattle breeds. Anim. Reprod. Sci. 15:61–79.

Bleul, U. and E. Gotz. 2013. The effect of lactic acidosis on the generation and compensation of mixed respiratory-metabolic acidosis in neonatal calves. Vet. Rec. 172:528.

Burfeind, O. and W. Heuwieser. 2012. Validation of handheld meters to measure blood L-lactate concentration in dairy cows and calves. J. Dairy Sci. 95:6449-6456.

Burfeind, O., V. S. Suthar, R. Voigtsberger, S. Bonk, and W. Heuwieser. 2011. Validity of prepartum changes in vaginal and rectal temperature to predict calving in dairy cows. J. Dairy Sci. 94:5053–5061.

Caroprese, M., M. Albenzio, A. Marzano, L. Schena, G. Annicchiarico, and A. Sevi. 2010. Relationship between cortisol response to stress and behavior, immune profile, and production performance of dairy ewes. J. Dairy Sci. 93:2395–2403.

Chen, Y., R. Arsenault, S. Napper, and P. Griebel. 2015. Models and Methods to Investigate Acute Stress Responses in Cattle. Animals: an Open Access Journal from MDPI 5:1268-1295.

Consitt, L. A., G. Saxena, A. Saneda, and J. A. Houmard. 2016. Age-related impairments in skeletal muscle PDH phosphorylation and plasma lactate are indicative of metabolic inflexibility and the effects of exercise training. Am. J. Physiol. Endocrinol. And Metab. 311:145-156.

Dubuc, J., T. F. Duffield, K. E. Leslie, J. S. Walton, and S. J. LeBlanc. 2010. Risk factors for postpartum uterine diseases in dairy cows. J. Dairy Sci. 93:5764–5771.

El-Tarabany, M. S. 2015. Effects of calving difficulty on the subsequent reproductive performance and milk production of Holstein, Brown Swiss and their crosses. Livest. Sci. 180:263-267.

EMA, The European Agency for the Evaluation of Medicinal Products. 1997. Denaverine Hydrochloride Summary Report.

Henningsen, G., H. Marien, W. Hasseler, M. Feldmann, H. A. Schoon, M. Hoedemaker, and K. Herzog. 2017. Evaluation of the iVET® birth monitoring system in primiparous dairy heifers. Theriogenology 102:44-47.

Herfen, K. and H. Bostedt. 1999. Correlation between clinical and laboratory diagnostic evaluation of the vitality of newborn calves under particular consideration of length and type of parturition. Wien Tierarztl. Monat 86:255-261.

Homerosky, E. R., N. A. Caulkett, E. Timsit, E. A. Pajor, J. P. Kastelic, and M. C. Windeyer. 2017. Clinical indicators of blood gas disturbances, elevated L-lactate concentration and other abnormal blood parameters in newborn beef calves. Vet. J. 219:49-57.

Hydbring, E., A. Madej, E. MacDonald, G. Drugge-Boholm, B. Berglund, and K. Olsson. 1999. Hormonal changes during parturition in heifers and goats are related to the phases and severity of labour. J. Endocrinol. 160:75–85.

Karapinar, T., O. Kaynar, A. Hayirli, and M. Kom. 2013. Evaluation of 4 point-of-care units for the determination of blood l-lactate concentration in cattle. J. Vet. Intern. Med. 27:1596-1603.

Kovács, L., F. L. Kézér, and O. Szenci. 2016. Effect of calving process on the outcomes of delivery and postpartum health of dairy cows with unassisted and assisted calvings. J. Dairy Sci. 99:7568-7573.

Kovacs, L., F. L. Kezer, E. Albert, F. Ruff, and O. Szenci. 2017. Seasonal and maternal effects on acid-base, l-lactate, electrolyte, and hematological status of 205 dairy calves born to eutocic dams. J. Dairy Sci. 100:7534–7543.

Jensen, M. B. 2012. Behaviour around the time of calving in dairy cows. Appl. Anim. Behav. Sci. 139:195–202.

Johanson, J. M. and P. J. Berger. 2003. Birth weight as a predictor of calving ease and perinatal mortality in Holstein cattle. J. Dairy Sci. 86:3745-3755.

Lombard, J. E., F. B. Garry, S. M. Tomlinson, and L. P. Garber. 2007. Impacts of dystocia on health and survival of dairy calves. J. Dairy Sci. 90:1751-1760.

Mainau, E., and X. Manteca. 2011. Pain and discomfort caused by parturition in cows and sows. Appl. Anim. Behav. Sci. 35:241–251.

Mee, J. F. 2004. Managing the dairy cow at calving time. Vet. Clin. North Am. Food Anim. Pract. 20:521–546.

Meyer, C. L., P. J. Berger, and K. J. Koehler. 2000. Interactions among Factors Affecting Stillbirths in Holstein Cattle in the United States1. J. Dairy Sci. 83:2657-2663.

Miedema, H. M., M. S. Cockram, C. M. Dwyer, and A. I. Macrae. 2011a. Behavioural predictors of the start of normal and dystocic calving in dairy cows and heifers. Appl. Anim. Behav. Sci. 132:14-19.

Miedema, H. M., M. S. Cockram, C. M. Dwyer, and A. I. Macrae. 2011b. Changes in the behaviour of dairy cows during the 24 h before normal calving compared with behaviour during late pregnancy. Appl. Anim. Behav. Sci. 131:8-14.

Mormède, P., S. Andanson, B. Aupérin, B. Beerda, D. Guémené, J. Malmkvist, X. Manteca, G. Manteuffel, P. Prunet, C. G. van Reenen, S. Richard, and I. Veissier. 2007. Exploration of the hypothalamic–pituitary–adrenal function as a tool to evaluate animal welfare. Physiol. Behav. 92:317–339.

Mülling, M. 1977. Asphyxie des neugeborenen Kalbes. Prakt. Tierarzt 58:78-80.

Nagel, C., J. Aurich, L. Trenk, N. Ille, M. Drillich, W. Pohl, and C. Aurich. 2016a. Stress response and cardiac activity of term and preterm calves in the perinatal period. Theriogenology 86:1498-1505.

Nagel, C., L. Trenk, C. Aurich, N. Ille, M. Pichler, M. Drillich, W. Pohl, and J. Aurich. 2016. Sympathoadrenal balance and physiological stress response in cattle at spontaneous and PGF(2 alpha)-induced calving. Theriogenology 85:979-985.

Noakes, D. E., T. J. Parkinson, and G. C. W. England. 2001. Parturition and the care of parturient animals. Pages 155–187 in Arthur's Veterinary Reproduction and Obstetrics. 8th ed. W.B. Saunders, Oxford, UK.

Ouellet, V., E. Vasseur, W. Heuwieser, O. Burfeind, X. Maldague, and E. Charbonneau. 2016. Evaluation of calving indicators measured by automated monitoring devices to predict the onset of calving in Holstein dairy cows. J. Dairy Sci. 99:1539-1548.

Pahl, C., E. Hartung, A. Grothmann, K. Mahlkow-Nerge, and A. Haeussermann. 2014. Rumination activity of dairy cows in the 24 hours before and after calving. J. Dairy Sci. 97:6935-6941.

Probo, M., A. Giordano, P. Moretti, G. Opsomer, L. O. Fiems, and M. C. Veronesi. 2012. Mode of delivery is associated with different hematological profiles in the newborn calf. Theriogenology 77:865-872.

Proudfoot, K. L., M. B. Jensen, P. M. Heegaard, and M. A. von Keyserlingk. 2013. Effect of moving dairy cows at different stages of labor on behavior during parturition. J. Dairy Sci. 96:1638-1646.

Richter, J., and D. Ahlers. 1993. Tiergeburtshilfe. Paul Parey, Berlin, Germany.

Rutten, C. J., C. Kamphuis, H. Hogeveen, K. Huijps, M. Nielen, and W. Steeneveld. 2017. Sensor data on cow activity, rumination, and ear temperature improve prediction of the start of calving in dairy cows. Comput. Electron. Agr. 132:108-118.

Saint-Dizier, M., and S. Chastant-Maillard. 2015. Methods and onfarm devices to predict calving time in cattle. Vet. J. 205:349–356.

Schuenemann, G. M., I. Nieto, S. Bas, K. N. Galvao, and J. Workman. 2011. Assessment of calving progress and reference times for obstetric intervention during dystocia in Holstein dairy cows. J. Dairy Sci. 94:5494-5501.

Shah, K. D., T. Nakao, and H. Kubota. 2006. Plasma estrone sulphate (E1S) and estradiol-17 beta (E-2 beta) profiles during pregnancy and their relationship with the relaxation of sacrosciatic ligament, and prediction of calving time in Holstein-Friesian cattle. Anim. Reprod. Sci. 95:38-53.

Sorge, U., D. Kelton, and R. Staufenbiel. 2009. Neonatal blood lactate concentration and calf morbidity. Vet. Rec. 164:533-534.

Stewart, M., J. R. Webster, G. A. Verkerk, A. L. Schaefer, J. J. Colyn, and K. J. Stafford. 2007. Non-invasive measurement of stress in dairy cows using infrared thermography. Physiol. Behav. 92:520-525.

Streyl, D., C. Sauter-Louis, A. Braunert, D. Lange, F. Weber, and H. Zerbe. 2011. Establishment of a standard operating procedure for predicting the time of calving in cattle. J. Vet. Sci. 12:177–185.

Tenhagen, B. A., A. Helmbold, and W. Heuwieser. 2007. Effect of various degrees of dystocia in dairy cattle on calf viability, milk production, fertility and culling. J. Vet. Med. A Physiol. Pathol. Clin. Med. 54:98–102.

Vannucchi, C. I., J. A. Rodrigues, L. C. Silva, C. F. Lucio, and G. A. Veiga. 2015. Effect of dystocia and treatment with oxytocin on neonatal calf vitality and acid-base, electrolyte and haematological status. Vet. J. 203:228-232.

Veronesi, M. C. 2016. Assessment of canine neonatal viability-the Apgar score. Reprod. Domest. Anim. 1:46-50.

Veyx-Pharma Gmb, H. 2009. Fachinformation Sensiblex 40 mg/mL. (Zulassungsnummer 3100278.00.00.) Veyx-Pharma GmbH, Schwarzenborn, Germany.

Villettaz Robichaud, M. V., D. L. Pearl, S. M. Godden, S. J. LeBlanc, and D. B. Haley. 2017a. Systematic early obstetrical assistance at calving: I. Effects on dairy calf stillbirth, vigor, and passive immunity transfer. J. Dairy Sci. 100:691–702.

Villettaz Robichaud, M. V., D. L. Pearl, S. M. Godden, J. Rushen, S. J. LeBlanc, and D. B. Haley. 2017b. Systematic early obstetrical assistance at calving: II. Effects on dairy heifer calf growth, health, and survival to weaning. J. Dairy Sci. 100:703–712.

von Borell, E., J. Langbein, G. Despres, S. Hansen, C. Leterrier, J. Marchant-Forde, R. Marchant-Forde, M. Minero, E. Mohr, A. Prunier, D. Valance, and I. Veissier. 2007. Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals—A review. Physiol. Behav. 92:293–316.

Wehrend, A., K. B. Padberg, and H. Bostedt. 2003. Electronic quantification of traction force during use of a mechanical calving aid in bovine parturition. Berl. Munch. Tierarztl. Wochenschr. 116:12–19.

Zehner, N., J. J. Niederhauser, M. Schick, and C. Umstatter. 2019. Development and validation of a predictive model for calving time based on sensor measurements of ingestive behavior in dairy cows. Comput. Electron. Agr 161:62-71.

Zobel, R. and J. Taponen. 2014. Denaverine hydrochloride and carbetocin increased welfare during and after parturition and enhanced subsequent fertility in cattle. J Dairy Sci. 97:3570-3577.

7 PUBLICATIONS

Research Papers:

Lange, K.; C. Fischer-Tenhagen and W. Heuwieser (2017):

Predicting stage 2 of calving in Holstein-Friesian heifers. Journal of Dairy Science. 100:4847–4856

Lange, K.; W. Heuwieser and C. Fischer-Tenhagen (2018):

Effect of denaverine hydrochloride application to heifers on the APGAR score and lactate concentration in newborn calves. Tierärztliche Praxis Großtiere. 46:150-153

Lange, K.; W. Heuwieser and C. Fischer-Tenhagen (2019):

Influence of denaverine hydrochloride on calving ease in Holstein-Friesian heifers. Journal of Dairy Science. 102:5410-5418

Oral presentation at conference:

Lange, K.; Fischer-Tenhagen, C.; Heuwieser, W. (2016):

Predicting the onset of stage two of calving in Holstein-Friesian heifers. 9. Doktorandensyposium & DRS Präsentationsseminar "Biomedical Sciences" Berlin – 16.09.-16.09.2016.n: 9. Doktorandensyposium & DRS Präsentationsseminar "Biomedical Sciences" Berlin: Mensch und Buch Verlag, S. 23 ISBN: 978-3-86387-744-6

Poster presentation at conference:

Lange, K.; Fischer-Tenhagen, C.; Heuwieser, W. (2016):

Predicting the onset of stage two of calving in Holstein-Friesian heifers. 29th World Buiatrics Congress Dublin, Irland – 03.07.-08.07.2016. In: The 29th World Buiatrics Congress, Dublin 2016 - Congress Proceedings – Michael Doherty (Hrsg.) Dublin, Irland: Veterinary Ireland 13 The Courtyard, Kilcarbery Park, Nangor Road, Dublin 22 S. 570–571 ISBN: 978-1-5262-0432-5

8 ACKNOWLEDGEMENTS

Mein erster Dank gilt Herrn Prof. Dr. Heuwieser, der mir dieses spannende und praxisnahe Thema überlassen hat. Ich bin dankbar für Ihre gute, strukturierte wissenschaftliche Anleitung, Ihre konstruktive Kritik und die Möglichkeit eigene Ideen umzusetzen. Vielen Dank, dass ich die Gelegenheit hatte, in die Welt der Nutztierethologie zu schnuppern. Dem Verein Tiergyn e.V. danke ich für die finanzielle Unterstützung.

Als nächstes ein ganz herzliches Dankeschön an meine Betreuerin Dr. Carola Fischer-Tenhagen, die mich immer durch die anspruchsvolle Zeit der Doktorarbeit begleitet hat. Danke für deine vielen guten Ideen, deinen Bezug zur Praxis und deine Unterstützung in der Umsetzung. Danke, dass du immer ansprechbar bei Fragen warst und das Ziel nie aus den Augen verloren hast.

Ein weiterer herzlicher Dank gilt dem gesamten Team der Tierklinik für Fortpflanzung, insbesondere meinen Büromitbewohnerinnen Julia und Joana, der Kaffeegesellschaft, sowie dem Team Hunderunde. Danke für eine spannende Zeit in einer Klinik fernab der ‚Heimatuni', die mir unglaublich viele neue Eindrücke beschert hat. Danke für die vielen neuen und kreativen Anregungen, wie man Lehre fortschrittlicher gestalten kann und neue wissenschaftliche Erkenntnisse umsetzt. Außerdem wäre die Zeit ohne euch nicht annähernd so amüsant gewesen und ohne eure Unterstützung, in jeder Hinsicht, wäre diese Arbeit nicht gelungen.

Ohne die menschlichen und tierischen Mitarbeiter der teilnehmenden Betriebe hätte dieses Projekt niemals stattfinden können. Danke für euer Vertrauen und die großartige Zusammenarbeit!

Danke an die Klinik für Rinder der Tierärztlichen Hochschule Hannover, samt ihren Mitarbeitern und Patienten, die meine Begeisterung für Kühe und Buiatrik geweckt haben.

Zuletzt bin ich dankbar für die Unterstützung meiner Familie, meines Partners und meiner Freunde. Ihr habt mich immer begleitet, ermutigt und motiviert. Danke dafür!

9 DECLARATION OF INDEPENDENCE

Hiermit erkläre ich, dass ich, Katrin Lange, alle Studien selbständig durchgeführt und die vorliegende Arbeit selbständig angefertigt habe. Ich versichere, dass ich ausschließlich die angegebenen Quellen und Hilfen in Anspruch genommen habe.

Tabelle 1. Eigener Anteil[1] an den Forschungsprojekten der vorliegenden Dissertation

	Studie 1[a]	Studie 2[b]	Studie 3[c]
Studienplanung	+++	+++	+++
Datenerhebung	+++	+++	+++
Datenanalyse	+++	+++	+++
Verfassen des Manuskripts	+++	+++	+++
Editieren des Manuskripts	++	++	++

[1]Legende: +++: > 70%

++: 50−70%

+: < 50%

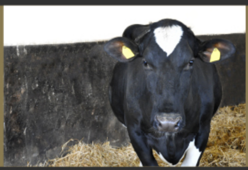
[a] Predicting stage 2 of calving in Holstein-Friesian heifers

[b] Effect of denaverine hydrochloride application to heifers on the APGAR score and lactate concentration in newborn calves

[c] Influence of denaverine hydrochloride on calving ease in Holstein-Friesian heifers

Berlin, den 21.09.2020

Katrin Lange



Dissertation Freie Universität Berlin
Druck: Mensch und Buch Verlag