Aus dem Zentrum für klinische Tiermedizin der Tierärztlichen Fakultät der Ludwig-Maximilians-Universität München

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Establishment of a standard operating procedure for predicting the time of calving in cattle

Inaugural-Dissertation zur Erlangung der tiermedizinischen Doktorwürde der Tierärztlichen Fakultät der Ludwig-Maximilians-Universität München

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Meiner geliebten Frau

Wenn wir neue Fragen stellen –

werden wir neue Antworten bekommen

Janus & Jokisch

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List of abbreviations

ACTH	Adrenocorticotrophic hormone
AI	Artificial insemination
AUC	Area under the curve
BCS	Body condition score
C.l.	Corpus luteum
CAP	Contraction associating protein
EIA	Enzyme immune assay
h	Hours
HF	Holstein-Friesian
HPA	Hypothalamus-pituitary-adrenal axis
ICC	Intra observer correlation coefficient
IOR	Inter observer reliability
P4	Progesterone
PGE_2	Prostaglandin E2
$PGF_{2\alpha}$	Prostaglandin F 2 α
PRBT	Progesterone rapid blood test
PS	Parturition score
PS-PRBT	Combination of PS and PRBT
PTGS2	Prostaglandin synthetase II
ROC	Receiver operating characteristics
SOP	Standard operating procedure

I INTRODUCTION

In accordance with the Farm Animal Welfare Council (FAWC, 2009), our responsibilities as veterinarians are, among others, to ensure that the right of “freedom from pain, injury or disease” is ensured using “prevention or by rapid diagnosis and treatment”.

A large number of different works recently demonstrated that calf viability after dystocia is significantly decreased (MEE, 2004; TENHAGEN et al., 2007; TAVERNE & NOAKES, 2009). Depending on the severity of the dystocic situation, dams have increasingly worse complications, such as endometritis (TENHAGEN et al., 2007; MEE, 2008), and in the worst cases, increased mortality rate (MCGUIRK et al., 2007; TENHAGEN et al., 2007). Therefore, early recognition of calving animals enable the farmer and veterinarian to diagnose and treat dystocic situations early to prevent severe consequences for both the calf and the cow.

Management and observation of calving cows and heifers is crucial for calf viability and dam health (MEE, 2004). Numerous studies (BERGLUND et al., 1987; PARKER et al., 1988; STEVENSON, 1989; LAMMOGLIA et al., 1997; AOKI et al., 2005; HOFMANN et al., 2006) have attempted to optimise this process through the examination of the change in clinical signs during the preparatory stage of calving. However, the reports about the success of these attempts in the field were contradictory.

The parameter, with the best sensitivity and sensibility, changing before calving is a drop in progesterone (P4) in the peripheral blood 12 to 48 h before parturition (PARKER et al., 1988; MATSAS et al., 1992; REXHA & GRUNERT, 1993; BIRGEL et al., 1994) . However, analysis of blood before calving is either too expensive and/or time-consuming. Additionally, the use of on-farm progesterone rapid tests has not yet been established successfully for a pre-partum cow, most likely due to the high financial impact of investigating all calving animals.

The aim of this study is to develop a standardised method for the prediction and exclusion of parturition in cows and heifers within the next 12 h after examination. The standardised method is addressed to individual animals of special interest, e.g. cows with a severe underlying disease or a history of dystocia in the past, because in these cases, a more intensive monitoring of calving animals is needed.

First, the predictive values of seven clinical parameters were investigated individually. These parameters included relaxation of the broad pelvic ligaments, relaxation of the tail, physiological hyperplasia of the udder, oedema of the udder, filling of the teats, oedema of the vulva, and discharge of vaginal mucous. Additionally different combinations of clinical parameters were investigated to elucidate their combined predictive values. For the standardised calving prediction, the best clinical parameters and most predictive combination were chosen.

In the second part of this work, the sensitivity and specificity of a commercially available progesterone rapid blood test (PRBT), already validated for cycling cows, was used to analyse the progesterone in the last few days of gestation to estimate the impact of using this test in addition to observation of aforementioned clinical parameters.

The third part of this study determined, whether a standard operating procedure for prediction or exclusion of calving within 12 h could be created, based on a combination of different clinical findings and a PRBT as additional tool in cases the clinical findings were ambiguous.

1 Physiology of parturition

In cattle, parturition occurs after a physiological gestation length of 270 to 290 days, which is dependent on the breed (RICHTER & GÖTZE, 1993).

1.1 Endocrinological aspects of parturition

The main source of P4 production in cattle is the corpus luteum (C.l.) graviditatis. During the gestation period the palcenta only produces sufficient amount of P4 from day 150 to 200. This was demonstrated, because of the gestational maintenance in ovariectomised cattle in this period due to sufficient placental P4 production (MCDONALD et al., 1953; HOFFMANN & SCHULER, 2002). However, for parturition, a decline in P4, caused by regression of the C.l., is essential for physiological calving. The direct regression is initiated by the release of prostaglandin$F_{2\alpha}$ ($PGF_{2\alpha}$). The endocrinological changes responsible for its release were best observed in the sheep, but the results in sheep are probably very similar to the changes occurring in cows.
Initiation of parturition results from the activation of the foetal hypothalamus-pituitary-adrenal (HPA) axis. Reasons for its activation have not yet been elucidated, but different possibilities have been discussed, including maturation of the foetal hypothalamus; response of the foetal hypothalamus to placental hormones; and foetal stressors due to hypoxia, hypocapnia, changes in blood pressure or blood glucose (WOOD, 1999). When the HPA is activated, adrenocorticotrophic hormone (ACTH) is released by the pituitary along with a simultaneous increase in the binding capacity of plasma for cortisol. These factors in turn reduce the amount of free cortisol in plasma, thus decreasing the negative feedback effect of the ACTH to the pituitary. Additionally, the response rate of the foetal adrenal gland increases with foetal age (GLICKMAN & CHALLIS, 1980). The increase in the foetal cortisol concentration induces intrauterine prostaglandin synthesis by two different pathways (WHITTLE et al., 2000): an oestrogen-dependent pathway and an oestrogen-independent pathway. In the oestrogen-independent pathway, cortisol enhances expression of prostaglandin synthetase II (PTGS2) within the foetal trophoblast cells of the placenta. In this mechanism, prostaglandin E_2 (PGE_2) is released in the placenta, stimulating the conversion of pregnenolone to C19 steroids. P4 is then aromatised by 17a-hydroxylase and aromatase to oestrogens (WHITTLE et al., 2000). This increase in oestrogen levels causes activation of PTGS2, among other enzymes, in the maternal endometrium, which increases the production and release of $PGF_{2\alpha}$ (WHITTLE et al., 2006; SCHAUB et al., 2008). There are various effects of $PGF_{2\alpha}$: $PGF_{2\alpha}$ lowers the threshold of the myometrial oxytocin receptors, indirectly increasing myometrial contractions and directly contracting the uterine smooth muscle (TAVERNE & NOAKES, 2009). Another effect of increasing $PGF_{2\alpha}$ production in cows and goats is induction of C.l. regression. Therefore, prostaglandins are the main initiating factors of parturition and are essential for initiating smooth muscle contractions (EILER et al., 1984; FUCHS et al., 1995; VAN ENGELEN et al., 2007).
The oestrogen also has a direct impact on the myometrium by increasing the amount of contraction

associating proteins (CAPs) (OLSON, 2003; MENDELSON, 2009), which increases myometrial cell connections, by forming gap junctions to coordinate myometrial contractions, oxytocin and prostaglandin receptors and calcium channels. Another essential effect resulting from the presence of oestrogens is the softening of the birth canal (namely the cervix, the vagina and the associated tissues by altering the structure of collagen fibres). Additionally, oestrogens increase vascular permeability, shown clinically by oedemas at different locations, e.g., the udder (JANOWSKI et al., 2002) and the vulva.

1.2 Stages of labour (German doctrine)

In the literature, two different doctrines on the different stages of labour that occur during physiological calving have been established. The German doctrine describes five different stages (1.2) and the Anglo-Saxon (1.3) doctrine describes three different stages.

1.2.1 The preparatory stage

The first stage of labour begins approximately two to three weeks before parturition. The first stage of labour is a passive stage because it is dominated by hormonal changes, namely, increasing oestrogen levels in the peripheral blood (SHAH et al., 2006; SHAH et al., 2007) and decreasing P4 levels in the last 48 h before parturition (PARKER et al., 1988; MATSAS et al., 1992; REXHA & GRUNERT, 1993; BIRGEL et al., 1994). The clinical characteristics, which are due to the hormonal changes, are dominated by the softening of the ligaments at the pelvis (SHAH et al., 2006), relaxation of the tail, and by hyperaemia and oedema of the birth canal and the udder. The physiological hyperplasia of the udder occurs in the last one to two weeks prior to parturition and the filling of teats in the last few days before calving (BIRGEL et al., 1994). An increase in mucous production of the vaginal and cervical mucus membranes follow, clinically visual as mucous discharge (HOFMANN et al., 2006). In the last two to three days before calving, the clinical signs increase dramatically, especially the relaxations of the broad pelvic ligaments, so that they are not palpable anymore (BIRGEL et al., 1994).

1.2.2 The opening stage

The opening stage is divided into two phases. The first, the passive phase, is characterised by the opening of the cervix (BREEVELD-DWARKASIN et al., 2002; TAVERNE et al., 2002; WEHREND et al., 2004), which is due to a decrease in the muscular tonus of smooth muscle cells. The opening of the cervix starts at the ostium cervicale externum and extends toward the ostium cervicale internum. The second phase of this stage, the active phase, is characterised by the initial contractions of the uterus. The contractions start when the cervical canal is completely open, at a diameter of 5 to 7 cm (RICHTER & GÖTZE, 1993). Cows show clinical signs such as impatience or paddling only in the active phase. In the opening stage, contractions press the allantois and the amnion sac against the cervix (TAVERNE et al., 2002). The opening stage, which lasts 6 to 16 h, ends with the rupture of the allantois sac. Clinically, this stage is only recognised by visual confirmation of the allantois sac: a blue shiny colour seen in the vulva. Therefore, the beginning of the opening stage cannot currently be determined

(GRUNERT & ANDRESEN, 1996).

1.2.3 The dilatation stage

The dilatation stage, first defined by WALTHER & MARX (1957), begins with the rupture of the allantois sack and ends, in the forward position of the calf, when the front of the calf has passed through the vulva. In the case of a backward position, the dilatation stage ends when the pelvis of the calf has passed through the vulva. The time duration of this stage depends on the number of gestations. For pluripara in cows, the dilatation stage is one to three hours long; in primipara, the dilatation stage is four to six hours long. This stage is characterised by the abdominal press, which occurs due to pelvic reflex, because the calf triggers pressure receptors at the vaginal roof (RICHTER & GÖTZE, 1993).

1.2.4 The expulsion stage

This stage begins by the passing of the front of the calf through the vulva (GRUNERT & ANDRESEN, 1996) and ends with the total expulsion of the calf out of the uterus. The total time duration of this stage varies in the literature between five and forty minutes (GRUNERT & ANDRESEN, 1996; BOSTEDT, 2003; WEHREND et al., 2005). The dam normally rests for up to one to two minutes after the front of the calf passes the vulva before complete expulsion of the calf occurs.

1.2.5 The stage of placental expulsion

The last stage of the labour, the expulsion of the secundinae, is another important part of parturition. The physiological length of this stage varies in the literature [reviewed by (MCNAUGHTON & MURRAY, 2009; BEAGLEY et al., 2010)] between 6 to 24 h. After expulsion of the calf, contractions of the uterus expulse the foetal membrane without any abdominal press.

1.3 Stages of labour (Anglo-Saxon doctrine)

The first stage of labour

The changes and actions that occur during the first stage of labour cannot be observed externally because this stage includes the dilatation process of the cervix, the onset of myometrial contractions and the foetus entering the birth canal. All these processes are caused by the hormonal changes described above (see Chapter 1.1), such as the onset of myometrial contractions following the decrease in P4 and the increase in oestrogens, which in turn, increases CAPs and $PGF_{2\alpha}$, which have a direct effect on the myometrium (TAVERNE & NOAKES, 2009). The first stage of labour ends with appearance of at least one foetal claw or the allantois sack in the vulva.

The second stage of labour

In the second stage of labour, the expulsion of the foetus occurs. This stage starts with the appearance of the calf, and is characterised by the abdominal press; therefore, it can be recognised externally. The dominating processes of this stage are the coordination of myometrial contractions and the abdominal press because both are essential for the foetal expulsion (TAVERNE & NOAKES, 2009). This stage ends with the delivery of the calf.

The third stage of labour

The third stage of labour is identical to the stage of placental expulsion described above in Chapter 1.2.5.

1.4 Partus prediction procedure

In all domestic animals, forecasting parturition is very important for optimising the monitoring of parturition.

1.4.1 Cattle

In the last few decades, numerous investigations have been conducted to optimise calving prediction. The well-known characteristic signs of cows in the preparatory stage are:

- relaxation of the broad pelvic ligaments,
- physiological hyperplasia of the udder,
- oedema of the udder,
- filling of the teats,
- oedema of the vulva,
- relaxation of the vulva,
- vaginal mucous,
- restlessness,
- change in body temperature.

These parameters were investigated in several studies. The results of these studies in predicting calving were, in part, very contradictory. Some authors were able to demonstrate a high predictive value of some of these clinical signs (BERGLUND et al., 1987; BIRGEL et al., 1994; SHAH et al., 2007), with special emphasis on the relaxation of the broad pelvic ligaments. The predictive value of the hyperplasia of the udder was also a valuable observation in the work of BERGLUND et al. (1987) but not in other publications. In contrast to these works, other researchers found that the variation of the clinical signs were too great to obtain any valuable information (REXHA & GRUNERT, 1993; HOFMANN et al., 2006). The drop in body temperature, measured rectally and vaginally showed a predicitable trend in several studies, but continous measurements of the cow and the environmetal temperature were necessary to obtain useful information (AOKI et al., 2005; DUFTY, 1971; BIRGEL et al., 1994; LAMMOGLIA et al., 1997). In contrast, no predictive value of using the body temperature to forecast the time of calving was determined in the work of REXHA & GRUNERT (1993).

In contrast to the clinical results, different authors obtained the same results in monitoring the drop in P4 before calving (PARKER et al., 1988; MATSAS et al., 1992; REXHA & GRUNERT, 1993; BIRGEL et al., 1994). Different P4 on-farm tests were also analysed by different authors with very similar results (PARKER et al., 1988; MATSAS et al., 1992; REXHA & GRUNERT, 1993).

1.4.2 Other species

Mare

It is extremely difficult to recognise any signs of ongoing parturition in a mare. Because of strong gluteal muscles, no relaxation of the broad pelvic ligaments can be observed. The values of other clinical signs are also very poor. Therefore, research in the few last decades focused on analyses of pre-partal mammary secretion and the changes of its composition with special emphasis on changes in calcium carbonate concentrations. However, in the literature, these results are contradictory (LEY et al., 1993; LEY, 1994; DOUGLAS et al., 2002).

Sow

The physiological hyperplasia of the udder of a sow occurs a couple days before parturition. However, only just before or during intra-parturition is it possible to milk sows. In one study, shortly before parturition, sows refused to feed (RICHTER & GÖTZE, 1993). Additionally, an increase of 0.4°C body temperature 4 h before parturition was shown to be a good predictive factor in sows (HENDRIX et al., 1978).

Bitch

The observed factors in a bitch for predicting parturition within 24 to 48 h are quite noticeable. Particularly, the use of P4 enzyme-linked immunosorbent assays (ELISA) are very sensitive and a validated method for predicting parturition [reviewed by (LEROYER et al., 2002; KIM et al., 2007)] because a drop in P4 occurs several hours before parturition. Another possibility in predicting the time of parturition in bitches is through constant measurement of the body temperature; typically, the body temperature decreases one week before parturition from 39°C to 37.5°C and decreases by another degree immediately before parturition begins (CONCANNON et al., 1977).

2 Dystocia and its economic effects

The term “dystocia” comes from the Greek words “dys”, meaning “difficult”, and “tokos”, meaning “birth”. Dystocia has many different definitions and meanings throughout the world; therefore, the data on the incidence and the outcome of dytocia are sometimes contradictory. In general, dystocia can be defined as calving difficulty resulting in prolonged spontaneous, prolonged or rather severe assisted extraction (MEE, 2008), whereby the definition of these terms vary between observers. In our opinion, every aberrance from the physiological parturition is defined as a dystocia, either a prolonged spontaneous calving or a calving requiring any kind of assistance during parturition.

2.1 Reasons for dystocia

The reason for dystocia can either be in the dam or in the foetus. The different situations are described in Figure 1, but three main different complexes can be considered responsible for dystocia:

- expulsion factors,
- adequacy of the birth canal,

- size and disposition of the birth canal

In cases in which one or more of these problems occur, the cow will suffer from dystocia. Thus, obstetrical help is needed to protect the health of both the dam and the calf (TAVERNE & NOAKES, 2009).

2.2 Incidence of dystocia

Dystocia rates in industrial countries vary between 2 to 7% in cows and heifers. The U.S. was not included in these results because the prevalence of dystocia in the U.S. is around 13% (MEE, 2008). Reasons for this difference are most likely due to the fact that in the U.S., in contrast to most other countries, no rigorous selection for calving ease and dystocia reduction has been performed in the last few decades (MEE, 2008). Data on the incidence of dystocia must be reviewed very critically due to the different definitions of dystocia between different investigations. This problem can also be observed in the very contradictory data of calving assistance rates (10% to >50%) (HANSEN et al., 2004; ICBF, 2006; HERINGSTAD et al., 2007) and dystocia rates (5.5%) (ICBF, 2006).

2.3 Economic effects

The tremendous economic consequences of dystocia are numerous (MEE, 2004; MCGUIRK et al., 2007; TENHAGEN et al., 2007; TAVERNE & NOAKES, 2009):

- increased stillbirth rate,
- increased neonatal mortality,
- increased mortality rate of the dam,
- reduced productivity of the dam,
- reduced subsequent fertility and increased chance of sterility in the dam,
- increased likelihood of puerperal disease in the dam,
- increased likelihood of subsequent culling in the dam,
- veterinary costs.

In the last few decades, the sensitivity of the economic consequences of dystocia have increased, which is the reason for the high number of investigations recently performed in this field. The total financial impact of dystocia varies a lot from country to country as well as between various investigations. In the U.K., investigations in the last decade (MCGUIRK et al., 2007) showed that the total cost was dependent on the severity of dystocia. In the cases of slight dystocia, the total costs were up to £110, almost four times less than cases of severe dystocia (£400). The consequences of dystocia are dependent on its severity. In case control studies, effects concerning the reproductive performance, culling rate, milk yield and calf viability were correlated to the severity of dystocia (TENHAGEN et al., 2007).

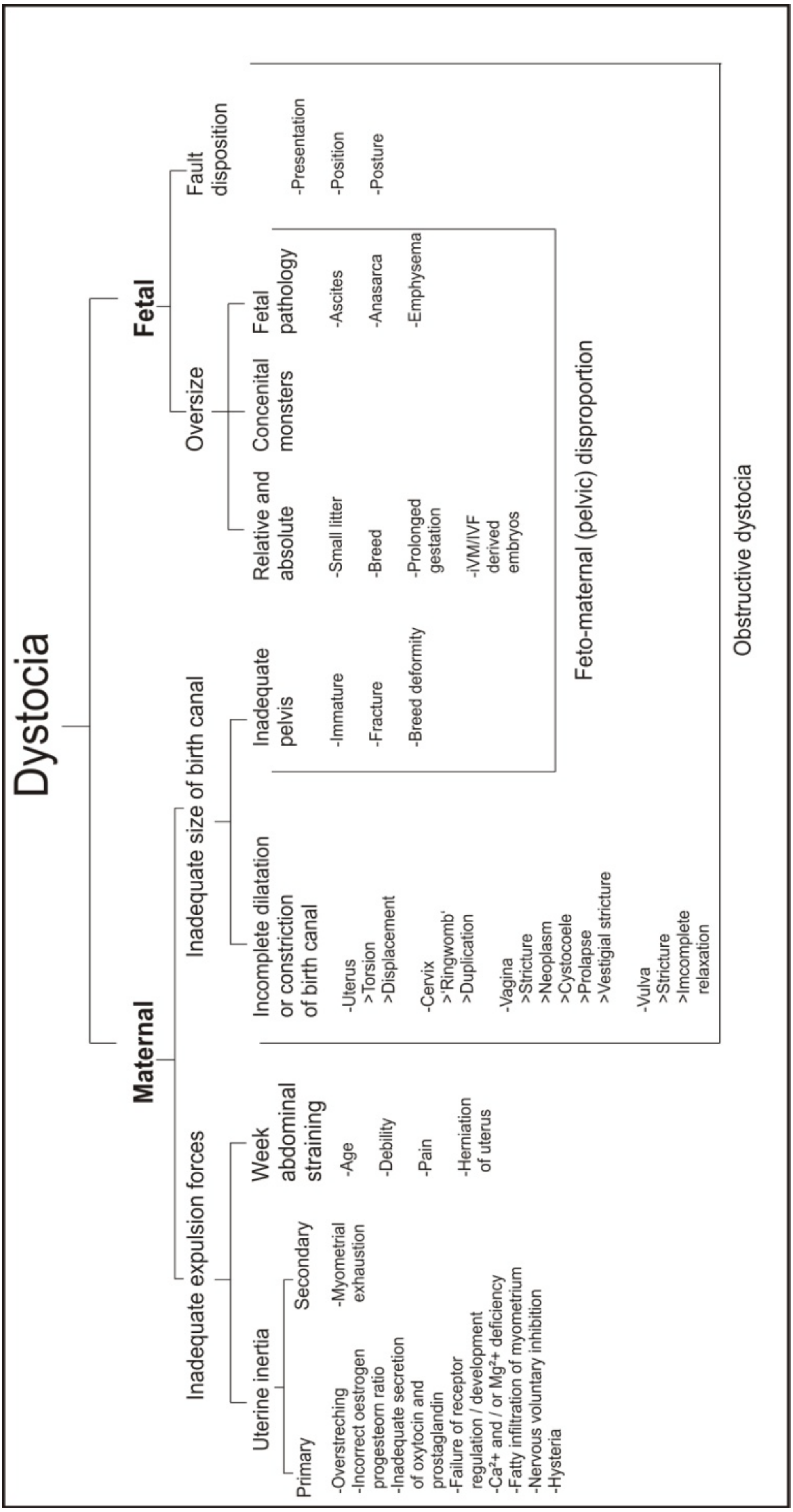

Figure 1: The causes of dystocia (adapted from (TAVERNE & NOAKES, 2009))

II RESULTS

1 Publication

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Original Article

Establishment of a standard operating procedure for predicting the time of calving in cattle

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Precise calving monitoring is essential for minimizing the effects of dystocia in cows and calves. We conducted two studies in healthy cows that compared seven clinical signs (broad pelvic ligaments relaxation, vaginal secretion, udder hyperplasia, udder edema, teat filling, tail relaxation, and vulva edema) alone and in combination in order to predict the time of parturition. The relaxation of the broad pelvic ligaments combined with teat filling gave the best values for predicting either calving or no calving within 12 h. For the proposed parturition score (PS), a threshold of 4 PS points was identified below which calving within the next 12 h could be ruled out with a probability of 99.3% in cows (95.5% in heifers). Above this threshold, intermitted calving monitoring every 3 h and a progesterone rapid blood test (PRBT) would be recommended. By combining the PS and PRBT (if PS ≥ 4), the prediction of calving within the next 12 h improved from 14.9% to 53.1%, and the probability of ruling out calving was 96.8%. The PRBT was compared to the results of an enzyme immunoassay (sensitivity, 90.2%; specificity, 74.9%). The standard operating procedure developed in this study that combines the PS and PRBT will enable veterinarians to rule out or predict calving within a 12 h period in cows with high accuracy under field conditions.

Keywords: cattle, forecasting, parturition, progesterone

Introduction

Calf mortality around parturition is highly associated with dystocia. In cases of severe parturition problems, calving mortality rates increase up to 50% [10,11]. Thus, predicting the time of calving is crucial for the health of newborn calves and their dams in difficult calving situations. Prediction also helps to prevent injuries to the newborn caused by the dam or the environment. For farm management, it is even more important to know if the cow is not likely to begin calving within 12 h because calving monitoring, a time-consuming process, would not be necessary. Calving monitoring is especially important for cows suffering from poor health along with primary labour insufficiencies as well as for cows with very valuable offspring (*e.g.,* calves produced by embryo transfer). Numerous researchers have attempted to develop methods for predicting parturition times more accurately as a key element for managing dairy cows [11].

Various physiological indicators have been utilised to predict the time of parturition with varying results. These parameters include changes in body temperatures, measured rectally as well as vaginally [1,3,5], and progesterone profiles [3,9]. In addition, the influence of external factors, such as climatological changes [4,16] or alteration in day length [6], on calving time have been investigated. Attempts have also been made to predict calving time based on individual external signs including relaxation of the pelvic ligaments [2,3], swelling of the vulva, and udder distension. It has been shown in a large number of cows that the presence of very relaxed ligaments indicates that parturition will probably occur within 24 to 72 h. However, studies performed up to now have always evaluated different external signs individually but not in combination as a method to predict the time of parturition. Therefore, in this study we investigated a combination of clinical signs that can be evaluated in the field with the aim of developing a useful and reliable method for predicting the time of calving within 12 h in cattle.

Progesterone, a hormone essential for pregnancy in all mammals, is produced by the corpus luteum (CL) and the placenta. It has been shown that a reduction in progesterone concentrations below 1.2 ng/mL is currently the most accurate way to predict calving time within 12 h [9]. Since quantitatively measuring progesterone levels is not practical in the field, we evaluated the sensitivity and specificity of a commercially available progesterone rapid

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blood test (PRBT). The objective of this study was to establish a new standard operating procedure (SOP) to accurately and easily predict the time of calving within 12 h. To do this, we used the PRBT in combination with an evaluation of clinical parameters that are thought to indicate parturition.

Materials and Methods

Two consecutive experiments were done for developing a validated SOP to predict whether or not calving will occur within the next 12 h. These studies were conducted according to the guidelines for ethical animal treatment, approved by the animal protection section of the district government of Upper Bavaria (Ref. No.: 55.2-1-54-2531.3-01306) and State Office of Frankfurt/Oder (Ref. No.: 23-2347-1-25-1-2009), Germany.

Experiment 1

A total of 21 clinically healthy cows of different breeds (14 Holstein-Friesian, six Simmental, and one Brown-Swiss) were used for Experiment 1. The animals were located at the Clinic for Ruminants of the University of Munich (three animals) as well as animals on two commercial farms at Bavaria and Brandenburg, respectively (18 animals); all animals were examined. Seven heifers and 14 pluripara cows were involved in Experiment 1. The animals at the Clinic for Ruminants were brought to the clinic, from their farms, located in southern Bavaria, at least 1 week prior to calving and housed tied up for calving monitoring. The animals on the two farms were housed untethered in cubical houses on straw bedding during the last weeks of gestation. All animals received an adequate total mix ration, out of grass silage, corn silage, hay minerals and concentrate and water during their gestation time.

For Experiment 1, an external obstetrical examination was conducted as previously described [3] once a day for at least 3 days before calving to assess external signs of the preparatory stage. The examinations were conducted at 8 a.m. by one veterinarian (DS). Table 1 presents an overview of the clinical signs that were evaluated. The alteration of each sign (broad pelvic ligaments relaxation, vaginal secretion, udder hyperplasia, udder edema, teat filling, tail relaxation, and vulva edema) during the preparatory stage to its maximum was divided in four steps (0; 1; 2; 3 points).

Experiment 2

The second experiment was conducted to verify the results obtained in Experiment 1, on one large dairy farm in Brandenburg. The average milk yield of this farm is 10,500 kg per year. In Experiment 2, a total of 124 healthy animals (90 cows and 34 heifers), which calved physiologically at 278 days (± 7 days) of gestation, were included. The

Table 1. Use of different clinical signs during the preparatory stage of cattle for a parturition scoring system

Clinical sign	PS-points			
Description	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%
Secretion of vaginal mucous[1]	None	Slight < 10 cm long; diameter < 1 cm	Moderate > 10 cm long; diameter < 1 cm	Extensive > 10 cm long; diameter > 1 cm
Physiological hyperplasia of the udder	Empty, small palpable	Slightly filled	Partially filled	Totally filled, enlarged, not palpable
Edema of the udder	None	On the base	Entire udder	Including the abdomen
Filling of the teats	Flaccid None	Slightly filled ~25%	moderately filled ~50%	completely filled ~100%
Relaxation of the tail*	No flexibility	45° ~ 90°	90° ~ 120°	120° ~ 180°
Edema of the vulva[†]	Strongly folded, no edema	Moderately folded, mild edema	Mildly folded, moderate edema	Not folded, high edema, redness of inner mucosa

This table is modified from Birgel *et al.* [3]. *The relaxation of the tail is tested by flexing the last third of the tail, [†]The tail has to be lifted to evaluate the vaginal mucous and edema of the vulva. The degree of flexure without any defence reaction should be estimated. PS: parturition score, PS-points: points of the PS.

animals, all Holstein-Friesian cows, were housed in their lactation period untethered in cubical houses with slatted floors while cows, after they were dried off, were housed with straw bedding in groups of different sizes. The animals received an adequate total mix ratio and water during their gestation time. The approximate body condition score of the animals at calving was 3.25. They were moved to a maternity pen approximately 5 days before calving. Milking cows were dried off approximately 6 to 7 weeks before the estimated day of calving.

External obstetric examinations were conducted once daily at least 3 days before calving. Following the results of Experiment 1 (Table 3), the caudal edge of the broad pelvic ligaments, edema in the vulva, relaxation of the tail, and the filling of the teats were examined. Additionally, body temperature was measured at 8:00 a.m. using a digital thermometer (Microlife, Switzerland). The thermometer measurement range was from 32.0 to 42.9°C with an accuracy of ± 0.1°C between 34°C and 42°C.

Collection of blood samples

Blood samples (5 mL with EDTA) were collected from the tail vein during all obstetrical examinations (with minimized immobilisation using a feeding rack) at least 3 days prior to parturition. The samples were centrifuged immediately (2,000 × g for 5 min), and the plasma was stored in 1 mL aliquots at −20°C until analysis.

Progesterone enzyme immunoassay (EIA)

Progesterone concentrations were determined by a competitive heterologous enzyme immunoassay as previously described [13] with minor modifications. Direct measurement of plasma progesterone levels was made using a rat anti-progesterone monoclonal antibody (Sigma Aldrich, USA). The sensitivity of the test in terms of the 50% intercept was 1 ng/mL. The lowest detectable concentration (significant different from zero: B/Bo − 2SD) was 0.2 ng/mL. All intra- and inter-assay variations were < 9.5%. Plasma samples (EDTA), from the examined cows, described above, were diluted 1:10 in assay buffer [40mM Na_2HPO_4 (Merck, USA); 0,14 M NaCl (pH 7.2; Merck, USA); 0,1% v/w bovine albumin fraction V (effective sample volume, 1 μL per well; Serva, Germany)]. Equipments were used to perform the EIA; Spectra Filter-Photometer (Tecan, Germany), MTP reader (Tecan, Germany), EasyFit software (Tecan, Germany) and Transferpette (Brand, Germany).

Progesterone rapid blood test (PBRT)

A commercially available semi-quantitative EIA kit (Hormonost Easy Rind; Biolab, Germany) was used to analyse blood plasma (after centrifugation) or serum (after blood coagulation with or without centrifugation). This test was conducted at the cow's side to detect CL activity, by following the manufacturer instructions. Briefly, 5 drops of serum were filled into coated test tubes (provided by the manufacturer) and 5 drops of dilution solution were added. 2 drops of enzyme marked progesterone were added and after 5 min of incubation (at room temperature) the test tubes were rinsed and 10 drops of substrate for the enzyme reaction were added. After five minutes the results were compared (visual assessment) to those of two controls (CL active = progesterone above 1 ng/mL; CL inactive = progesterone below 1 ng/mL), which were treated in parallel to the test samples.

Data analysis

Data were analysed using Microsoft Excel and PASW Statistics 18 (version 18.0.0; SPSS, USA). The sensitivity and specificity for predicting calving within 12 h were calculated for each clinical sign. Receiver operating characteristic (ROC) analyses were conducted to determine the optimal cut-off points of each individual clinical sign to distinguish between calving and the absence of calving within the next 12 h. The progress of the clinical signs was rated by assigning parturition score (PS)-points between 0 and 3 except for the broad pelvic ligament parameter which was assigned PS-points between 0 and 6 to give it double weighting. This takes into consideration the high reliability of this parameter, which was confirmed by the statistical analysis. For practical reasons, only those parameters that could be considered in combination in both standing and reclining animals were evaluated. These parameters were relaxation of the broad pelvic ligaments, filling of the teats, edema of the vulva, and relaxation of the tail. Combinations of these different clinical signs were also evaluated using ROC analysis. The inter observer reliability (IOR) and the intra observer correlation coefficients (ICC) were calculated for each of these four parameters to determine the comparability between different observers and the reproducibility of one observer.

For Experiment 1, 95% confidence intervals for specificity were calculated for each possible cut-off score (ranging from zero to the maximum). The purpose of predicting the calving time was to exclude the possibility of parturition within 12 h to avoid costs due to animal monitoring. Therefore, the optimum cut-off point was chosen when the upper confidence limit was still 100% and the confidence interval range was the smallest. Sensitivity and specificity were calculated for the most practical combination of clinical signs, progesterone EIA, and PRBT results. Additionally, ROC analyses of the different clinical signs and their sum were conducted to determine differences in their value. ROC analyses were also conducted to analyse differences between cows and heifers. Temperature data were analysed by a one-way ANOVA to determine differences in mean temperatures between time points.

Results

Experiment 1

Since the exactness of the different clinical signs used to predict calving is a precondition for a robust PS, ROC analysis was conducted for each individual clinical sign. The area under the curve (AUC) was used to rank the signs according to their ability to rule out or forecast calving. The best clinical indicator for calving within 12 h (Table 2) was relaxation of the pelvic ligaments (AUC = 0.775), followed by filling of the teats (AUC = 0.733). No differences were observed between heifers and cows (data not shown).

To increase the exactness of predicting the calving time, we analysed combinations of different clinical signs as described above. Due to its high AUC in the single clinical sign analysis and proven correlation to the time of partus, the "relaxation of pelvic ligaments" parameter was included in all scores. To find the best combination of signs, the AUC was calculated in the ROC analysis (Table 3). For all combinations of clinical signs, a double weighting (PS-points 0, 2, 4, and 6) of the "relaxation of pelvic ligaments" parameter resulted in higher AUCs than weighting this parameter with single PS-points (0, 1, 2, and 3) as assigned (Table 3). Triple PS-points (0, 3, 6, and 9) for this parameter did not yield higher AUC values (data not shown).

The 95% confidence intervals for the proportion of animals classified as not calving within 12 h (Table 3) were calculated for the different combinations of clinical signs. The combination with all four parameters had the smallest confidence interval meaning that this combination provided the best accuracy for ruling out calving within 12 h. The smallest confidence interval for specificity (excluding calving within 12 h) was achieved if the sum of the PS-points was less than seven. Then the PS ruled out calving within 12 h with a probability of 97.9% for all animals.

Table 2. The predictive value of individual clinical signs for predicting calving within 12 h (Experiment 1)

Clinical signs	AUC*
broad pelvic ligaments	0.775
teat filling	0.733
hyperplasia of the udder	0.732
vulva edema	0.666
tail relaxation	0.634
udder edema	0.624
mucous secretion	0.578

*Area under curve (AUC) was determined in the receiver operating characteristic (ROC) analysis.

Experiment 2

We calculated the AUC by ROC analysis of all four parameters with respect to their ability to predict "calving" or "no calving" within 12 h. The AUC for the relaxation of the tail and edema of the vulva ranged from 0.545 to 0.712 and from 0.526 to 0.692, respectively, in all cattle. Relaxation of the pelvic ligaments and filling of the teats had higher AUC values ranging from 0.729 to 0.856 and 0.644 to 0.780, respectively.

The IOR of the relaxation of the tail and the edema of the vulva were 0.32 and 0.42, respectively. The IOR of the relaxation of the pelvic ligaments and the filling of the teats had higher a kappa value of 0.86 and 0.82, respectively, in cows as well as heifers. The ICC for relaxation of the tail

Table 3. Results of a receiver operating characteristic (ROC) analysis and calculation of confidence intervals when using different combinations of clinical signs for calving prediction (Experiment 1)

Combinations of clinical signs	AUC	% of cattle not calving within 12 h	Confidence interval		
			Lower	Upper	Range*
PL + TR + TF + VO	0.811	99.0	94.0	100.0	6.0
PL + TR + TF	0.819	100.0	92.9	100.0	7.1
PL + TF	0.816	98.5	91.0	100.0	9.0
PL + TR	0.797	100.0	85.4	100.0	14.6
PL + TR + VO	0.790	100.0	83.9	100.0	16.1
PL + TF + VO	0.810	100.0	83.9	100.0	16.1
PL + VO	0.782	94.7	73.2	100.0	26.6

Clinical signs (Table 1) were used for calving prediction. PL were double weighted (PS-points 0, 2, 4, and 6) for these calculations; the scores for all other signs were used as described (PS-points: 0, 1, 2, and 3). ROC curves were determined for each combination and the AUC was calculated. PL: pelvic ligaments, TR: tail relaxation, TF: teat filling, VO: vulva edema. *Range: the difference between the upper and lower confidence interval due to PASW statistics.

and edema of the vulva were 0.15 and 0.05, respectively. The ICC for relaxation of the pelvic ligaments was 0.86 and 0.88 for filling of the teat.

The subsequent analyses focused on the sum of the scores for relaxation of the caudal edge of the broad pelvic ligaments (PS-points: 0, 2, 4, and 6) and filling of the teats (PS-points: 0, 1, 2, and 3). Analyses of the AUCs for cows versus heifers showed significant differences. Fig. 1 shows the ROC curve of all 124 animals as well as those for the groups of cows and heifers. The AUC of all animals was 0.835, the AUC of the cows 0.852, and that of the heifers 0.745.

To find the optimal cut-off point for distinguishing between "calving" and "no calving", the maximum value of the sum of sensitivity and specificity was chosen. This optimal cut-point was at 4.5 PS-points out of 9. Since only a whole number is possible for the PS, the predictive value was calculated for cut-off values of 4 as well as 5 PS-points. By using the PS for cows only, the prediction of "no calving" within the 12 h was 98.0% if the threshold was set at 5 (sensitivity: 79.0%; specificity: 78.7%) and the prediction of "calving" within 12 h was 22.4% (sensitivity: 79.0%; specificity: 78.7%). If the threshold was set at 4, the chance of "no calving" within the 12 h was accurately predicted in 99.3% of the cases (sensitivity: 94.7; specificity: 57.8%), and "calving" within 12 h was accurately predicted in 14.9% of the cows (sensitivity: 94.7%; specificity: 57.8%). In heifers, the predictive value of the PS was

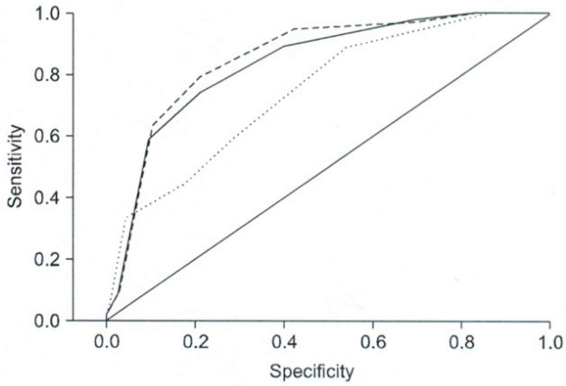

Fig. 1. Receiver operating characteristic (ROC) analysis of parturition score (PS)-points for examining the relationship between the combination of broad pelvic ligament relaxation and filling of the teats and the ability to predict calving within 12 h. All animals in this study (—) had an area under the curve (AUC) of 0.835. The cows (- - -) had an AUC of 0.852 and the heifers (····) had an AUC of 0.745. Straight diagonal line is reference line.

Table 4. Sensitivity, specificity, and predictive value of the parturition scores for predicting "calving" or "no calving" within 12 h

Cows and Heifers (n = 124)				Cows (n = 90)				Heifers (n = 34)			
Threshold PS-points	Calving within 12 h			Threshold PS-points	Calving within 12 h			Threshold PS-points	Calving within 12 h		
	Yes	No	Total		Yes	No	Total		Yes	No	Total
≥5	34	124	158	≥5	30	104	134	≥5	4	20	24
<5	12	464	476	<5	8	384	392	<5	5	93	98
	4	588	634		38	488	526		9	113	122
Sensitivity	73.9			Sensitivity	79.0			Sensitivity	44.4		
Specificity	78.9			Specificity	78.7			Specificity	82.3		
+Pred. value	21.5			+Pred. value	22.4			+Pred. value	16.7		
−Pred. value	97.5			−Pred. value	98.0			−Pred. value	94.9		
Threshold PS-points	Calving within 12 h			Threshold PS-points	Calving within 12 h			Threshold PS-points	Calving within 12 h		
	Yes	No	Total		Yes	No	Total		Yes	No	Total
≥4	41	235	276	≥4	36	206	242	≥4	5	29	34
<4	5	353	358	<4	2	282	284	<4	4	84	88
	46	588	634		38	488	526		9	113	122
Sensitivity	89.1			Sensitivity	94.7			Sensitivity	55.6		
Specificity	60.0			Specificity	57.8			Specificity	74.3		
+Pred. value	14.9			+Pred. value	14.9			+Pred. value	14.7		
−Pred. value	98.6			−Pred. value	99.3			−Pred. value	95.5		

Calculations were performed using the 5 and 4 PS-point thresholds for the PS. +Pred. value: positive predictive value; −Pred. value: negative predictive value.

different if a threshold of 4 was used (Table 4). It was possible to predict “no calving” with 95.5% accuracy (sensitivity: 55.6%; specificity: 74.3%) and the prediction of “calving” within 12 h was 14.7% accurate (sensitivity: 44.4%; specificity: 82.3%).

Body temperature

The mean average body temperature in prepartum cows and heifers declined by 0.3 ± 0.5°C from 38.9 to 38.6°C during the last 24 h before parturition. In 46.7% (n = 118) of the prepartum animals, a decline of >0.3°C was observed (Fig. 2) over the last 24 h before parturition. Body temperatures during the last 24 h before parturition were significantly different to those measured at all other time points ($p < 0.05$).

Quantitative analysis of prepartal progesterone levels

To observe the decrease in progesterone before parturition, a quantitative progesterone EIA was conducted. The reduction in progesterone levels (< 1.2 ng/mL) always occurred 36 to 12 h before parturition. With a sensitivity of 93.5% (specificity: 91.6%), progesterone values below 1.2 ng/mL were found to indicate the beginning of parturition within the next 12 h. On the other hand, progesterone serum concentrations were stable from 72 to 36 h *ante partum* (data not shown).

Evaluation of the PRBT in the calving preparatory stage

As shown above, the reduction in progesterone levels was the most precise and objective indication of calving. An on-farm PRBT for cycling cows was tested to see if it could be used as a predictive tool during the prepartal period. We compared this semi-quantitative PRBT with the standard quantitative progesterone EIA analysis. The PRBT had a sensitivity of 90.2% and a specificity of 74.9% for detecting progesterone levels higher or lower than 1.2 ng/mL (Fig. 3).

Partus prediction by using a combination of the PS and PRBT in cows: PS-PRBT

We developed an SOP that combines the evaluation of clinical PS and PRBT data (Fig. 4). When both methods

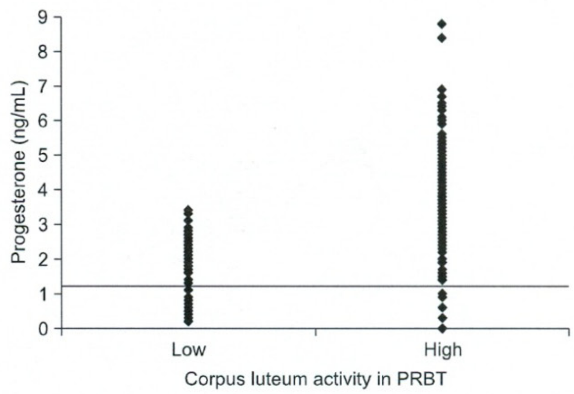

Fig. 3. Evaluation of a commercially available progesterone rapid blood test (PRBT) during the prepartum period. Progesterone was measured by an enzyme immunoassay as the gold standard and compared to semi-quantitative PRBT. The threshold progesterone level for an active corpus luteum (CL) is reported in the literature to be 1.2 ng/mL. The PRBT could differentiate between low (progesterone below 1 ng/mL) and high (progesterone above 1.2 ng/mL) CL activity. The sensitivity of the PRBT was 90.2% and the specificity 74.9%. Each diamond corresponds to a single blood sample.

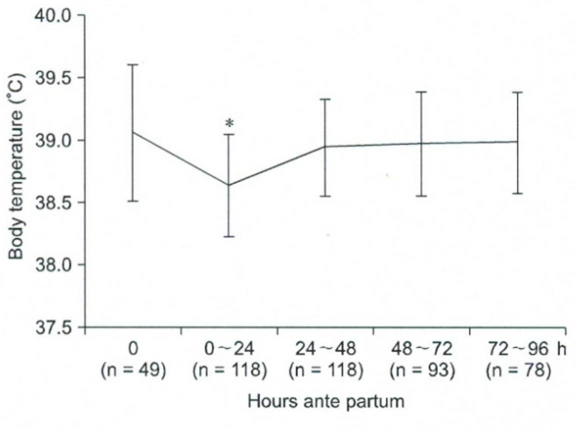

Fig. 2. Average body temperature of cattle during the last 4 days of gestation. The temperature in the last 24 h before parturition differed from all other time points (*$p < 0.05$). In the graph, 0 represents the time of calving (the number of animals investigated at that time point are in brackets).

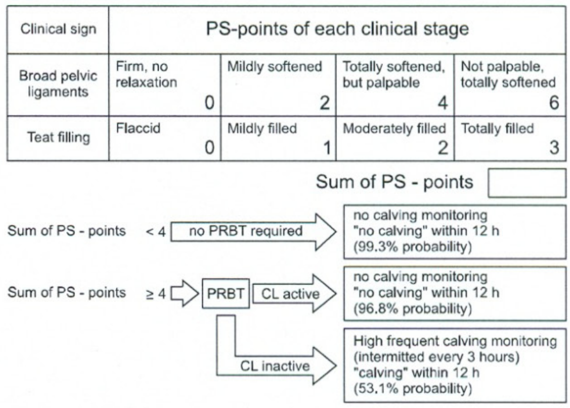

Fig. 4. Standard operating procedure of the PS-PRBT with a threshold of 4 PS-points. If the PS-PRBT is used with a threshold of 5 PS-points, the probability for “no calving” is 98.0%. If the PS-PRBT indicates an active CL, the probability of “no calving” is 93.3% and the probability for “calving within 12 h” is 65.8%.

Table 5. Sensitivity, specificity, and predictive value of the progesterone rapid blood test (PRBT) for cows with ≥ 5 PS-points or ≥ 4 PS-points (n = 54 cows) to predict "calving" and "no calving"

PS-points ≥ 5				PS-points ≥ 4			
PRBT	Progesterone (ng/mL)			PRBT	Progesterone (ng/mL)		
	≤1.2	>1.2	Total		≤1.2	>1.2	Total
Low	25	13	38	Low	34	30	64
High	3	42	45	High	3	91	94
	28	55	83		37	121	158
Sensitivity	89.3			Sensitivity	91.9		
Specificity	76.4			Specificity	75.2		
+Pred. value	65.8			+Pred. value	53.1		
−Pred. value	93.3			−Pred. value	96.8		

+Pred. value: positive predictive value; −Pred. value: negative predictive value. PS-points: results of the PS.

were combined, the following probabilities were calculated for cows: 93.3% for "no calving within 12 h" (high progesterone levels according to the PRBT) and 65.8% for "calving within 12 h" (low progesterone levels according to the PRBT) when the threshold was set at 5 points. With a threshold of 4 points, the probability of "no calving" was 96.8% and that for "calving" was 53.1% (Table 5).

Discussion

Preventing severe consequences of dystocia by professional calving management avoids injuries to the dam and protects the calf; the mortality of calves is highly correlated to severe calving problems [10,11]. An established SOP may help to increase the quality of calving monitoring and management. This would be particularly important for sick cows and those with previous calving problems as well as very valuable cows or offspring. Because of this we investigated in the presented study the value of different well-known clinical signs [3,5,12,14] for predicting calving times in dairy cows using a single examination. For the first time, we used a combination of individual clinical signs to yield a PS which increased the predictive value of the clinical examination.

There is conflicting information in the literature about the predictive value of body temperature. Different authors described a drop of at least 0.4°C within 22 h before parturition [3,5,8]. In contrast, another study [14] found that observed changes in body temperature within the last 36 to 24 h before parturition have no significant predictive value. However, body temperature must be monitored for at least 3 days before parturition, and it is not possible to give a predictive answer about parturition from a single examination. Additionally, it is unclear if the described decline in body temperature occurs equally in animals suffering from fever. In this study, all animals had an overall average physiological body temperature from 38.6 to 39.1°C during the prepartal phase. Only 46.7% of the animals showed a decline in body temperature of at least 0.3°C. The standard deviation between the animals was 0.5°C. Therefore, the change in body temperature before calving appears to be of little value for predicting calving within 24 h.

During the preparatory stage, the progression of each clinical sign is similar, and the ability to predict calving within the next 12 h is reflected in the ROC curve associated with each sign. The relaxation of the broad pelvic ligaments was the best individual predictive clinical parameter. These findings are similar to previously reported results by different research groups [2,3,5,14]. In the present study, the AUC of the pelvic ligaments was the highest of all individual clinical signs; therefore, double weight was given to this sign in the proposed scoring system. Using the same combinations of clinical parameters, the highest AUC was calculated using double weighing (0; 2; 4; 6 PS-points) of the pelvic ligament in contrast to single or triple weighting (data not shown).

The results of Experiment 2 were similar to those of Experiment 1 because none of the clinical signs on their own could be used to precisely forecast calving within 12 h. However, in contrast to Experiment 1, the precision of calving prediction did not increase by accounting for more than two clinical signs. During the last days of gestation, using clinical signs like the edema of the vulva did not increase prediction precision. Since using more than the two clinical parameters - relaxation of the broad pelvic ligaments and filling of the teats - did not increase the exactness of forecasting calving within 12 h, we concentrated our analyses on these two parameters only. Using more parameters also resulted in a greater variation which would lead to more difficulties in interpreting the results. Another important aspect of using these two clinical parameters is the strong reliability in repeated examinations and between different observers, reflected in the good results of the IOR and ICC, of the filling of the teats as well as the relaxation of the broad pelvic ligaments. In contrast the reliabilities, reflected in the bad results of the IOR and ICC of the two other parameters, edema of the vulva and relaxation of the tail, were poor.

The most important information for farm management, with special attention organizing farm duties and calving monitoring, is the ability to predict "no calving" within 12 h after examination. Therefore, the PS is an excellent tool to optimise calving management in cows because the ability to predict "no calving" in cows was as high as

99.3%. The results in heifers were not that good, because the predictive value of "no calving" within 12 h was only 95.0% .

For cows that might suffer from health problems associated with dystocia (such weak labour or hypocalcaemia) or highly productive animals for which farmers invest more in the offspring (*e.g.,* embryo transfer), prepartum monitoring is essential. In the literature, there are different data about the external signs of the preparatory stage in heifers versus cows. In dairy cattle, significant changes in specific clinical signs were observed among pluriparous cows [2] but these were found to have only little informative value. However, we could observe differences in the predictive values of calving or not within 12 h between pluriparous and primiparous animals. In contrast, differences were described [7] between cows and heifers among beef cattle. We found that the clinical signs we monitored were more accurate for predicting calving times in cows compared to heifers, indicating that the changes in clinical factors during the last days of the preparatory stage are less informative in heifers.

The predictive value of the PS for determining "no calving within 12 h" was 99.3% using a PS threshold of 4 PS-points (this was 98.0% with a threshold of 5 PS-points). We used the PRBT to increase these predictive values for animals with $\geq$ 4 PS-points (or $\geq$ 5 PS-points). The PRBT we used was originally developed as a method for early heat detection in cycling cows 19 to 21 days after insemination [15]. The precision of the test during the last days of gestation is similar to that in cycling cows. The PRBT is a very simple on-farm test that assays either plasma or serum in approximately 20 min; no additional equipment is necessary to run the test.

In cows, the ability of the PS-PRBT to predict either "calving" or "no calving" can change depending on the threshold that is used. In cases the threshold was set at 5, the PS-PRBT was able to rule out parturition within the next 12 h in 93.3% of the cases (progesterone levels were high according to the PRBT) and to predict "calving" within 12 h in 65.8% of cases (PRBT indicated that progesterone levels were low). If a threshold of 4 was used, "no calving" was predicted in 96.8% of the cases and "calving" was predicted in 53.1%. Hence, we recommend using the established SOP, described in the present study, with the threshold of 4 PS-Points to obtain the most important information for the farmer with a cow, *e.g.* suffering a severe mastitis ante partum. On one hand, the farmer obtains a higher security in ruling out calving within the next 12 h by using the threshold of 4 PS-points, but on the other hand it will probably increase the number of PRBT that has to be conducted (~50% higher costs). On the other hand, using the established SOP, which combines the clinical examination and the PRBT, with a threshold of 5 points reduces the testing costs because fewer animals have to be tested, but this reduces the ability to predict "no calving within 12 h".

Although it has been shown by different authors [9,12,14] that a reduction in progesterone below 1.2 ng/mL [9] is the most accurate indication for predicting the time of "calving", we do not advise to use the PRBT on its own as this technique is relatively expensive. The purpose of the present study was to primarily establish a method for predicting whether or not a cow will give birth within a certain time period by an external obstetrical examination. This procedure would be supplemented by a progesterone test to increase the precision of the PS.

The IOR values showed that the established SOP produced results that were similar between different observers. The ICC also demonstrated that the results from one veterinarian would be consistent. By following the developed SOP described in this study, veterinarians in the field will be able to predict the time of calving more precisely. The authors also advise veterinarians to apply the SOP in practice to achieve comparable and reproducible results. The SOP established in our study provides a validated method for determining whether calving within a 12 h time period is possible. This will help to improve calving monitoring and the management of individual cows (this technique will not be as useful for monitoring heifers) with health problems or a history of difficult births, and animals that have special genetic value. The SOP established in our study has to be further validated by a prospective study with healthy cows as well as cows suffering from health problems such as prepartum hypocalcaemia or ketosis.

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2 Investigations of the reliability of the tested clinical parameters by the inter observer reliability and intra observer correlation coefficients

To ensure the reliability between different observers of the established SOP, the inter-observer reliability (IOR) and the intra-observer correlation coefficients (ICC) were investigated. Both analyses are necessary for controlling the reproducibility of clinical parameters. High IOR and ICC values are both necessary to use this SOP in the field for repetitive examinations.

To analyse the IOR and the ICC, four parameters were investigated: teat filling, tail relaxation, vulva oedema and relaxation of the broad pelvic ligaments. IOR values, investigated by three different observers, showed contradictory results. On one hand, the clinical parameters of tail relaxation (0.324) and vulva oedema (0.415) showed poor correlation, but on the other hand, teat filling (0.822) and relaxation of the broad pelvic ligaments (0.864) showed a very strong correlation in the IOR (Table 6).

The results of the ICC were similar. The ICC of tail relaxation (0.148) and vulva oedema (0.048) was very poor and the results of teat filling (0.880) and relaxation of the broad pelvic ligaments (0.868) were very strong (Table 7).

The results of these analyses ensure that the SOP can be used by different investigators, and therefore, its application in the field is feasible. The combination of the relaxation of the broad pelvic ligaments and the filling of the teats not only showed a good predictive value, it was the most reliable both for a single investigator as well as between different investigators.

Table 6: Inter observer reliability (IOR) by analysing the inter item correlation. The results of three different independent investigators were analysed (n = 12 cows).

Inter observer reliability						
	Mean value	Minimum	Maximum	Area	Covariance	Number of Items
Teat filling	0.822	0.810	0.835	0.024	0.000	3
Tail relaxation	0.324	0.181	0.439	0.258	0.014	3
Vulva oedema	0.415	0.365	0.460	0.095	0.002	3
Relaxation of pelvic ligaments	0.864	0.852	0.870	0.019	0.000	3

Table 7: Intra observer correlation coefficients (ICC), analysing the intra item correlation. The results of three independent, blinded investigations of one investigator were analysed (n = 10 cows).

Intra observer correlation coefficients						
	Mean value	Minimum	Maximum	Area	Covariance	Number of Items
Teat filling	0.880	0.796	0.922	0.126	0.004	3
Tail relaxation	0.148	0.167	0.111	0.056	0.001	3
Vulva oedema	0.048	0.000	0.145	0.145	0.006	3
Relaxation of pelvic ligaments	0.868	0.802	1.000	0.198	0.010	3

III DISCUSSION

1 Conceptual considerations of calving monitoring

The monitoring of pre-partal cows and heifers is an essential requirement for decreasing mortality in newborn calves and for healthy and productive dams. The economic effects of dystocia in cattle are tremendous and losses up to £400 per case have been reported (MCGUIRK et al., 2007). These economic effects are mainly related to animal health and welfare, which is very difficult to calculate. The surviving calves after dystocia are of enhanced susceptibility to stillbirth rate and postnatal mortality. The dams often suffer from direct trauma by injuries that occur during calving, such as rupture of the mucous membranes of the soft birth canal, which can result in phlegmon of the pelvis. These animals are also predisposed to retention of foetal membranes that have known effects, such as clinical or toxical metritis and subsequent chronic endometritis. For both cows and heifers, the direct consequences of dystocia include an increased mortality rate and indirectly include decreased productivity and reduced fertility (including sterility), which often ends in an increased premature culling rate.

Therefore, the monitoring of calving animals was the focus of various previous studies. These studies concentrated only on single aspects (clinical symptoms or progesterone value) of the cow in the preparatory stage. However, they did not try to combine the different observations or symptoms for determining a single predictive outcome. For cows and heifers, typical clinical alterations occur in the last weeks and days of gestation, including the relaxation of the broad pelvic ligaments, physiological hyperplasia of the udder, oedema of the udder, filling of the teats, oedema of the vulva, relaxation of the vulva and vaginal mucous defluxion. All of these clinical changes are related to alterations in steroid hormones at the end of gestation. The predominant hormone during gestation in cattle is progesterone (P4), which is constantly produced by the Corpus luteum (C.l.) during pregnancy. At the end of gestation, foetal cortisol levels increase due to the activation of the hypothalamus-pituitary-adrenal axis. The cortisol activates a trophoblastic PTGS2, increasing PGE_2. This prostaglandin stimulates the conversion of pregnenolone to oestrogen. This cascade is responsible for increased oestrogen levels, which are measurable in peripheral blood (KANKOFER & MAJ, 1997). In addition to other effects, oestrogen increases vascular permeability, which can be observed clinically through the presence of different oedemas. The oedemas are mainly responsible for most findings, such as the relaxation of the broad pelvic ligaments. The role of relaxin in these processes is discussed very controversially in the literature [reviewed by (SHAH et al., 2006)].

The most objective and distinct parameter that changes in the preparatory stage is concentration of P4 in the peripheral blood; 12 to 48 h before calving, P4 drops below a threshold of 1 ng/ml (MATSAS, 1993; REXHA & GRUNERT, 1993).

In this thesis, the described changes in the preparatory stage of calving were investigated and predictive values for the prediction of the parturition within 12 h were calculated for each change. As a new step, the combination of the different parameters was examined to increase the predictive value of the clinical examination. Additionally, a progesterone rapid blood test (PRBT), only validated for cycling cows (SOBIRAJ et al., 1995), was used for the preparatory cows for monitoring an objective change (a drop in P4) in the pre-partal phase.

The developed standard operating procedure (SOP), which is described in this dissertation, mainly addresses veterinarians confronted with the question of selected animals on farms. The target animal group of the SOP is not the total herd. The SOP is addressed to animals of special interest, such as cows with a severe underlying disease or a history of dystocia in the past, because in these cases, a more intensive monitoring of calving animals is needed. It is important that the method is based on clinical examinations, which will only combine these findings with results of an on-farm progesterone test as an additional tool. This method will enable veterinarians and farmers to improve management of calving animals to avoid the severe consequences of dystocic calvings.

The following two chapters are focused on the discussion of the two main aspects of the study: the results of the obstetrical examination in context with previous studies and the use of the progesterone rapid test in cows in the last trimester of gestation. The other important aspects of this work are discussed in further detail in Chapter II1(see discussion of the publication).

2 What is the impact of the clinical examination in prediction of calving?

The parameters, investigated in this study, changing always in the preparatory stage, are well-known and have been investigated in the past (DUFTY, 1971; REXHA & GRUNERT, 1993; RICHTER & GÖTZE, 1993; BIRGEL et al., 1994; HOFMANN et al., 2006; TAVERNE & NOAKES, 2009):

- relaxation of the broad pelvic ligaments,
- relaxation of the tail,
- physiological hyperplasia of the udder,
- oedema of the udder,
- filling of the teats,
- oedema of the vulva,
- vaginal mucous secretion.

For the first time we demonstrated that the combination of clinical parameters into one score increases the predictive value of these parameters. In the past, these parameters were only considered individually and not in combination.

A large-scale experiment in Sweden, in which 493 parturitions over seven years were analysed, demonstrated that the total relaxation of the broad pelvic ligaments is the strongest clinical sign of ongoing calving within 12 h. They showed that in 85.2% of the cases, where the broad pelvic ligaments were totally relaxed, calving occurs within 12 h. However, in only 49.3% of all investigated animals, this total relaxation actually occurred (BERGLUND et al., 1987). Another study with 23 examined animals also demonstrated that the relaxation of the broad pelvic ligaments was the most valuable clinical sign. However, in that investigation, only 52.2% of all animals showed a strong relaxation 12 h before calving (BIRGEL et al., 1994). In the presented work, the relaxation of the broad pelvic ligaments was also the most valuable clinical sign, reflected by the largest area under the curve (AUC) of all investigated signs. In the last 24 h before calving, all investigated animals showed signs indicative of the combination of relaxation of the broad pelvic ligaments and filling of the teats. In 93.8% of the examined animals, a relaxation of the pelvic ligaments was recognised, and in 96.9% of the animals, filling of the teats was observed. These results demonstrate that the combination of the two parameters increases the predictive value of the SOP because a completely developed parameter is not necessary in this method, in contrast to the investigations described above.

The other parameters investigated by BERGLUND et al. (1987) and BIRGEL et al. (1994) such as distension of the udder, swelling of the vulva, discharge of the vulva, and leaking of colostrum, were of a low predictive value because in only approximately 50% of the animals these signs appeared before calving. These results compliment the presented results (Chapter, II.) and BIRGEL, et al. (1994) also investigated alterations of the udder, but they distinguished between the physiological hyperplasia, oedema of the udder and filling of the teats. The authors also observed differentiated evolution for each of these parameters, which is in-line with our findings, in which the predictive values of the different clinical parameters were hardly comparable to another. For example, they demonstrated that heifers exhibited an enlarged udder in addition to filled teats 84 h before parturition, but only 50% of cows developed this symptom 22 h before parturition and 85.7% 6 h before calving. In the case of the oedema of the udder, the differences were much bigger between heifers and cows (263 h vs. 37 h ante-partum). In the presented investigation, these findings are similar because we also found differences between heifers and cows in the predictive value of the clinical examination. Therefore, application in heifers is not advised regularly due to its higher insecurity. Because of the relatively small numbers of heifers, that were investigated, the calculation of the predictive values neither for “not calving” nor “calving” for the whole SOP was possible. Assumably they are also lower, than they are in cows.

The difference between the results of BERGLUND et al. (1987) and BIRGEL et al. (1994) as well as between BERGLUND et al. (1987) and the results of this study could be explained by the differences in the breeds due to their different intended purposes. BERGLUND et al. (1987) investigated Swedish Friesian, Swedish Red and White, Swedish Jersey and crossbreeds of these breeds. However, the results did not differentiate between these breeds. The differences between BIRGEL et al. (1994) and the results of this study may not be comparable because they investigated the German Black and White breed, a dual-purpose breed more comparable to the current German Simmental breed than to the single-purpose Holstein Friesian (HF), with its high U.S. HF impact, which we examined in this study. The proportion and focus on the high yield of HF cows is nationally and internationally still increasing because the need for milk- or beef-producing farmers is still necessary and will only increase in the future. Therefore, it will be necessary to validate the parturition score (PS) in different breeds to generalise the results for all breeds.

3 How does a progesterone test improve prediction of calving?

P4 is produced in cattle by the C.l. and during gestation additionally by the placenta. The presence of P4 during gestation is essential for its maintenance. In general, luteolysis is the physiological regression of the C.l. at the end of gestation, as described in detail in the Introduction (Chapter I1.1) and at Day 16/17 of the oestrus cycle. Luteolysis can be recognised by a drop in P4 below 1 ng/ml in the peripheral blood (MATSAS et al., 1992; REXHA & GRUNERT, 1993; TAVERNE et al., 2002), and 4 ng/ml in the milk (FRIGGENS & CHAGUNDA, 2005).

The physiological changes in the progesterone level during the oestrus cycle are utilised by different on-farm tests for early exclusion of pregnancy nineteen days after artificial insemination (AI) (FRIGGENS & CHAGUNDA, 2005; SONMEZ et al., 2007). These tests are developed for measuring P4 concentration in milk or blood. Normally, milk is used for P4 determination because it is much easier for the farmer to obtain. Because the chemical character of P4 is lipophilic, milk is enriched with a P4 concentration of up to 20 ng/ml during the oestrus cycle (FRIGGENS & CHAGUNDA, 2005). The prognostic value of the different tests is subject to great variations in the sensitivity and specificity of the tests (SOBIRAJ et al., 1995) and is also dependent on farm management. The commercial value for farm management should not be underestimated because, with an efficient system, the farmer can rebreed animals three weeks after unsuccessful AI, even when they are not showing heat.

Progesterone on-farm tests for blood, plasma or serum are less common. In the literature, there are only a few products described because for cycling dairy cows, it is much easier to use milk samples. In animals that are not milked, such as heifers, the use of such tests is rare.

Only three tests have been described in the literature, apart from ours, for cows at the end of gestation for application in parturition monitoring (PARKER et al., 1988; MATSAS et al., 1992; REXHA & GRUNERT, 1993). The descriptions of the sensitivity and specificity of these tests were heterogeneous due to different emphases described in these publications. PARKER et al. (1988) used *"Ovucheck Cowside, Cambridge Veterinary Science"* and investigated the progesterone levels in the pre-partum period and the application of an on-farm test in calving management. However, no further information concerning the accuracy of the test used was described. BIRGEL et al. (1994) evaluated the use of a progesterone rapid test for calving prediction, using *"Progesteron-Schnelltest Target™ Albrecht, Aulendorf/Württenberg"*, and concluded calving monitoring can be optimised using an on-farm progesterone test. However, they only provided information about the time period calving would occur dependent upon the results of the semi-quantitative test they used and no evaluation of the sensitivity and specificity of the test was described. Because of their focus on the P4 testing, multiple P4 tests were conducted over many days, which consumes a lot of time and/or money, thus dramatically decreasing the acceptance of such a method. Only MATSAS et al. (1992) describes the sensitivity (86.7%), specificity (90.8%), positive predictive value (75.0%) and negative predictive value (95.5%) of the *"CITE PROBE Semi-Quant™ Progesterone, Idexx Corp., Portland, ME"* test. A threshold of 2.0 ng/ml was set to indicate a low P4 level by using the described test. However, shortly before calving they demonstrated in the same publication that a P4 value of 1.2 ng/ml had the highest accuracy as an indicator of calving within 24 h. Because of this very high threshold, it may be necessary to repeat the test several times before calving occurs. Thus, the practicability of the *"CITE PROBE Semi-Quant^{TM} Progesterone"* may be reduced compared to the "*Hormonost$^{(R)}$, Biolab, München*" we used. Also, only the P4 on-farm test was evaluated and no combination inclusive of clinical parameters was used. The differences between the investigation of MATSAS et al. (1992) and our own, such as the difference in the P4 threshold, does not make a direct comparison easy. In the present study only animals were investigated with the *"Hormonost$^{(R)}$, Biolab, München"*, which were selected by clinical examination. An investigation is required in which both tests are compared directly. Unfortunately, the *"CITE PROBE Semi-Quant^{TM} Progesterone"* is not available anymore.

Only by the elimination or reduction of false positive and false negative results is an increase in the predictive value of the SOP possible. However, none of the tests described in the literature are available anymore, so their combined use with the clinical examination in the developed SOP is not possible. Therefore, more investigations and the development of a better on-farm blood test, which can use whole blood, serum or plasma, are necessary. Additionally, the evaluation of other commercially available on-farm tests, validated for cycling animals must be performed, because then it might be possible to increase the accuracy of the SOP.

4 What is the practical relevance and what are future prospects of the SOP?

The SOP was first and foremost established for veterinarians in the field who encounter animals of special interest or with special problems. As mentioned previously, the SOP was not established for calving monitoring on herd levels, but for individual animals. Veterinarians are consulted for calving prediction normally only in cases of sick animals, which are presumed only to calf with help or animals with a history of dystocia, because these animals are more likely to suffer from dystocia again. Another group of animals with a special need for intensive calving monitoring are cows or heifers after embryo transfer or cows with very valuable breeding.

In the described SOP, the veterinarians must decide which of the described thresholds should be used in the clinical examination. As described previously (Chapter II1; see results and discussion of the publication), a threshold of four PS-Points and five PS-Points are possible. Using the lower threshold in the clinical examination increases the certainty of "no calving within 12 h", but it also reduces the predictive value of "calving within 12 h". By using a larger threshold (five PS-points), the certainty of "no calving within12 h" decreases from 96.8% to 93.3%, but the predictive value of "calving within 12 h" increases. The decision of using the threshold of 4 PS-points goes hand-in-hand with increased expense and time consumption for using the PRBT in cases of the threshold of four PS-Points because it is more likely that the PRBT has to be conducted more frequently. Under typical circumstances on the farm, a threshold of five PS-points is recommended because the acceptance of the farmer to perform the test is very important and is likely to be reduced by having to perform the test repeatedly. However, the conflict must be discussed by the veterinarians with their clients to find the best decision for every case.

In an exemplary cost calculation, the cost of the SOP, which must be performed two to three times, is approximately 5€ per test plus the veterinary cost. This cost must be compared to the economic loss in consequence of dystocia, e.g., to the cost of a dead calf and/or a cow with acute puerperal disorders and reduced fertility (150€ to 450€, adapted from MCGUIRK (2007)).

It is essential that the results of the SOP are comparable between different persons conducting the test because it is essential that a veterinarian can use the results of his or her colleague to correctly judge the development in the preparatory stage. Realistically, especially on week-ends, different veterinarians may care for the animals. Therefore, the reliability of the clinical examination must be very good between two independent observers (Chapter II.2).

For use in calving prediction, the current SOP can be improved in the future. Therefore, it is necessary to improve the sensitivity and specificity of the on-farm progesterone test that uses blood, serum or plasma. No improvements can be made in the clinical examination, so that the only way of improving the predictive value of the SOP is to reduce false positive results.

Another aspect for future work will be calving monitoring systems at the herd level, which would be of enormous economic benefit for farmers. Farmers can lose up to 450€ per animal suffering with dystocia (MCGUIRK et al., 2007). Thus, a standard procedure that is time- and cost-reducing for calving monitoring in herds would be highly beneficial. The easiest method, already used sporadically, is video camera-based monitoring of the calving paddock. However, data processing-based systems for video monitoring are conceivable and, similar to methods in oestrus control in which the activity of the cows in heat were measured, it might be possible to use the described restlessness of animals that occurs shortly before calving (BIRGEL et al., 1994) to calculate the movements and activity patterns of cows in the preparatory stage of calving. The use of infrared cameras could also be used for monitoring and computer-based recognition of the rupture of the allantois and amniotic sack through an increase of temperature around the cow caused by the release of the placental fluids.

IV SUMMARY

Establishment of a standard operating procedure for predicting the time of calving in cattle

Dominik Streyl

Precise calving monitoring is essential for decreasing the consequences of dystocia in cows and calves. The progress of seven clinical signs (broad pelvic ligaments relaxation, vaginal secretion, udder hyperplasia, udder oedema, teat filling, tail relaxation, and vulva oedema) in the preparatory stage were evaluated in two studies on healthy cows (n = 104) and heifers (n = 41) for the investigation of their suitability alone and in combination to predict the time of parturition. The animals were examined during (at least) the last three days before calving on a daily basis at 8:00 a.m. A commercial progesterone rapid blood test (PRBT) was used as an additional tool for calving prediction, which was compared to a validated enzyme immune assay.

The parturition score (PS) combines the sum of the PS-points of the relaxation of the broad pelvic ligaments (PS-points: 0; 2; 4; 6) and filling of the teats (PS-points: 0; 1; 2; 3) because these signs reported the best predictive value of all examined signs and the highest inter- and intra-observer reliability. In the PS, two thresholds of PS–points were defined (PS ≥ 4 and PS ≥ 5). Below PS 4 calving within 12 h could be ruled out with a probability of 99.3% in cows (95.5% in heifers). If a PS ≥ 5 is used calving within 12 h can be ruled out with a probability of 98.0% in cows (94.9% in heifers).

Above this threshold, application of the PRBT (sensitivity: 90.2%; specificity: 74.9%) was recommended. If the PRBT indicates an inactive corpus luteum, intermitted calving monitoring every three hours is recommended. By combining the PS and PRBT (if PS ≥ 4), the prediction of calving within the next 12 h improved from 14.9% (PS alone) to 53.1% and the probability of ruling out calving was 96.8%. If a PS ≥ 5 is used the probability to predict calving within the next 12 h is 65.8% and the probability of ruling out calving 93.3%.

The developed standard operating procedure that combines PS and PRBT will enable veterinarians the ability to rule out or predict calving within the next 12 h in peripartal animals with a relatively high accuracy under field conditions with only one single examination.

V ZUSAMMENFASSUNG

Etablierung eines Standardverfahrens für die Vorhersage der Geburt bei Kühen

Dominik Streyl

Eine gute Überwachung präpartaler Kühe ist essential, um die gravierendsten Folgen von Schwergeburten für Kuh und Kalb zu verhindern. Die Veränderungen von sieben typischen klinischen Anzeichen der nahenden Geburt (Erschlaffung des kaudalen Randes der breiten Beckenbänder, Schleimabgang aus der Vulva, Aufeutern, Euterödem, Füllung der Zitzen, Flexibilität des Schwanzendes und Vulvaödem) wurden in zwei Studien bei gesunden Kühen (n = 104) und Färsen (n = 41) untersucht. Ziel war es, ihren Nutzen, alleine oder in Kombination, zur Geburtsvorhersage zu evaluieren. Die Tiere wurden täglich mindestens über die letzten drei Trächtigkeitstage klinisch untersucht. Des Weiteren wurde ein kommerzieller Blut-Progesteron Schnelltest (BPST) mit Hilfe eines Enzymimmunoassays validiert und der Einsatz für die Geburtsvorhersage geprüft.

Der in der Arbeit entwickelte Geburtsscore (PS) kombiniert die Bewertung der Erschlaffung der breiten Beckenbänder (Punkte: 0; 2; 4; 6) und der Zitzenfüllung (Punkte: 0; 1; 2; 3). Diese beiden klinischen Parameter wiesen zum einen den höchsten prädiktiven Wert aller untersuchten Parameter auf. Zum anderen konnte für sie die höchste Verlässlichkeit sowohl zwischen zwei Untersuchern (Inter observer reliability (IOR)) als auch bei wiederholten Untersuchungen (Intra observer correlation coefficients (ICC)) gezeigt werden.

Für den PS wurde ein Grenzwert von 4 bzw. 5 Punkten in der klinischen Bewertung festgestellt. Unterhalb von 4 Punkten konnte eine Kalbung innerhalb der nächsten 12 h mit einer Wahrscheinlichkeit von 99,3 % bei Kühen und 95,5 % bei Färsen ausgeschlossen werden. Bei 5 Punkten betrug diese Wahrscheinlichkeit 98,0 % bei Kühen bzw. 94,3% bei Färsen. Oberhalb des Grenzwertes wird der Einsatz des BPST (Sensitivität: 90,2 %; Spezifität: 74,9 %) empfohlen. Im Falle des Testergebnisse „Progesteron niedrig“ wird zu intensiver Geburtsüberwachung geraten. Durch den kombinierten Einsatz des Geburtsscores (Grenzwert 4 Punkte) mit dem BPST kann der Geburtsbeginn innerhalb der nächsten 12 h mit großer Sicherheit ausgeschlossen werden (96,6%). Die Sicherheit der Vorhersage des Eintritts der Geburt in den nächsten 12h beträgt bei PS<4 Punkte und einem BPST „Progesteron niedrig“ 53,1%. Entsprechend kann bei einem Grenzwert von 5 Punkten im Geburtsscore die Kalbung mit einer Wahrscheinlichkeit von 65,8% für die nächsten 12 h vorhergesagt bzw. zu 93,3% ausgeschlossen werden.

Das in der vorliegenden Arbeit entwickelte Standardverfahren ermöglicht eine verbesserte Geburtsüberwachung und –vorhersage für Landwirte und Tierärzte unter Praxisbedingungen. Gegenwärtig eignet sich das Verfahren sehr gut für spezielle Geburtskühe (Problemkühe und Tiere mit hohem Wert), weniger für einen routinemäßigen Einsatz in der Gesamtherde.

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