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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 / Description	PS-points 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	$45^o \sim 90^o$	$90^o \sim 120^o$	$120^o \sim 180^o$
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

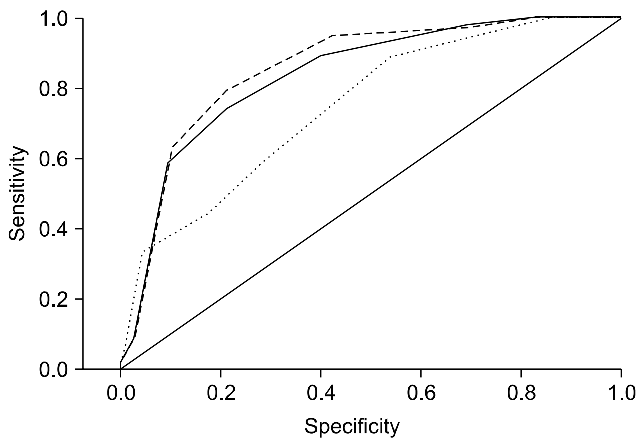

Fig. 1. Receiver operating characteristic (ROC) analysis of parturition score (PS)-points for examining the relationship between the combination of broad pelvic ligament relaxation and filling of the teats and the ability to predict calving within 12 h. All animals in this study (—) had an area under 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

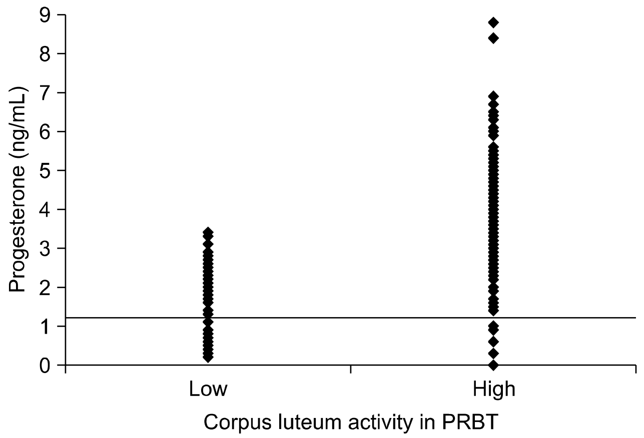

Fig. 3. Evaluation of a commercially available progesterone rapid blood test (PRBT) during the prepartum period. Progesterone was measured by an enzyme immunoassay as the gold standard and compared to semi-quantitative PRBT. The threshold progesterone level for an active corpus luteum (CL) is reported 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.

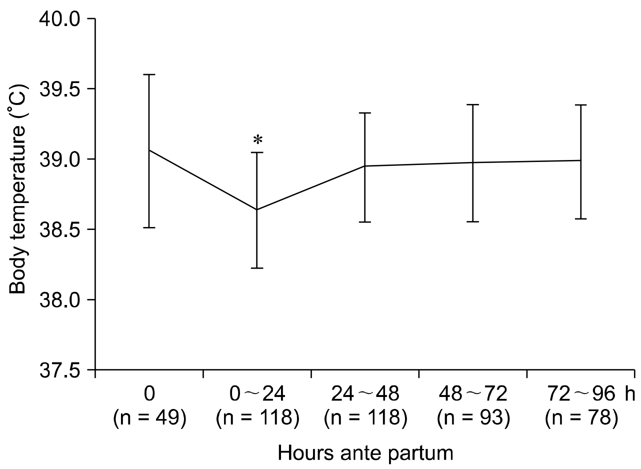

Fig. 2. Average body temperature of cattle during the last 4 days of gestation. The temperature in the last 24 h before parturition differed from all other time points (*$p < 0.05$). In the graph, 0 represents the time of calving (the number of animals investigated at that time point are in brackets).

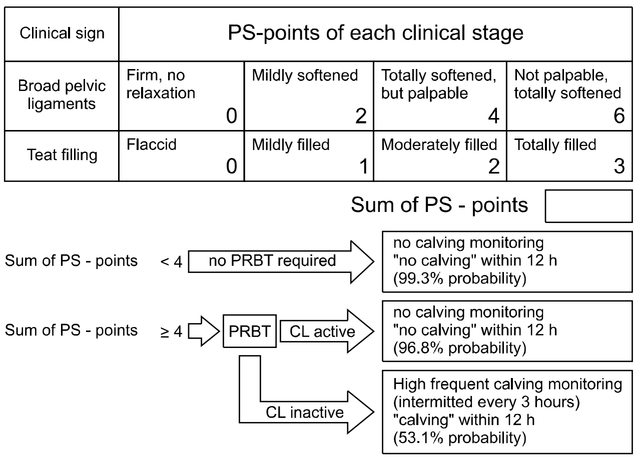

Fig. 4. Standard operating procedure of the PS-PRBT with a threshold of 4 PS-points. If the PS-PRBT is used with a threshold of 5 PS-points, the probability for "no calving" is 98.0%. If the PS-PRBT indicates an active CL, the probability of "no calving" is 93.3% and the probability for "calving within 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 ($\sim$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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