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2	Assessment of the temperature cut-off point by a commercial intravaginal device to predict
3	parturition in Piedmontese beef cows
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## 15 Abstract:

1

Dystocic parturitions have an adverse impact on animal productivity and therefore the profitability 16 of the farm. In this regard, accurate prediction of calving is essential since it allows for efficient and 17 prompt assistance of the dam and the calf. Numerous approaches to predict parturition have been 18 19 studied, among these, measurement of intravaginal temperature (IVT) is the most effective method at the field level. Thus, objectives of this experiment were, 1) to find an IVT cut-off to predict 20 calving within 24 h, and 2) to clarify the use of IVT as an automated method of calving detection in 21 housed beef cows. A commercial intravaginal electronic device (Medria Vel'Phone®) with a sensor 22 that measures the IVT every 12 h was used. Piedmontese cows (n = 211; 27 primiparous and 184 23 24 multiparous) were included in this study. One-way analysis of variance was used to assess the temperature differences at 0, 12, 24, 36, 48 and 60 h before parturition. Receiving operator 25 characteristic curves were built to determine the temperature cut-off which predicts calving within 26 27 24 h with the highest summation of sensitivity (Se) and specificity (Sp). Binomial logistic

regression models were computed to identify factors that may affect the IVT before calving. Mean 28 gestation length was  $291.5 \pm 13.7$  d (primiparous,  $292 \pm 14.1$  d; multiparous,  $289\pm9.2$  d). A 29 decrease (P < 0.001) in the average IVT was found from 60 h before calving until the expulsion of 30 the IVT device. A significant (P < 0.05) reduction in the IVT was noticeable from 24 h before until 31 parturition. The IVT drop to predict parturition 24 h before calving was 0.21°C (area under the 32 curve [AUC] = 0.72; Se = 66%, Sp = 76%). Furthermore, the IVT cut-off value to predict 33 parturition within 24 h was  $38.2^{\circ}$ C (AUC = 0.89; Se = 86%, Sp = 91%). None of the evaluated 34 fixed effects (parity, dystocia, season or length of gestation) affected (P > 0.05) the IVT variation 35 from 60 h before and up to calving. To conclude, the IVT average seems to be a better parameter 36 than the drop in temperature to predict parturition within 24 h. In this regard, a cut-off of 38.2°C 37 38 showed a high Se and Sp for predicting calving. This study demonstrates the usefulness of a commercially available device to predict calving to improve management in stabled beef farms. 39

40 **Keyword:** Intravaginal temperature, dystocia, prediction of parturition, partum assistance, cows.

# 41 **1- Introduction**

Parturition is a complex physiological event characterized by distinct physical and hormonal 42 changes, observable up to 72 h before parturition. Both dystocia and stillbirth have a significant 43 44 impact on animal productivity and the profitability of the farm; often requiring a skilled assistant and immediate intervention at the moment of delivery [1]. Moreover, dystocia has an adverse effect 45 on future reproductive performance and cattle welfare with increased incidences of retained 46 placenta (RP), uterine infections, and involuntary culling [2]. Patterson et al. [3] described dystocia 47 as the primary cause of calf mortality in the first 96 h of life. Human assistance at parturition is 48 49 reported to occur only between 11 and 51% of parturitions in beef and dairy cattle [4, 5, 6, 7]. When prompt and efficient assistance was achieved, a significant decrease in calf mortality, (RP), and 50 postpartum infections were reported [8]. Therefore, accurate calving prediction is a determining 51 52 factor to support animal welfare and herd profitability [9] and accurate calving management is crucial to reduce the adverse effects of dystocia for both the cow and the calf [12]. However, the 53

broad variability in gestation length and the unclear identification of the beginning of parturition
affect the possibility of prompt calving assistance [10, 11].

Numerous methods to predict parturition have been presented in literature over the years. Some 56 authors propose methods based on the measurement of external clinical signs of the preparatory 57 stage of parturition such as pelvic relaxation, vaginal secretion and udder hyperplasia [13]. Other 58 59 authors focused on measurement of the hormonal concentration of progesterone [14, 12] or estrone sulfate/17ß estradiol in blood [15]. Ultrasound monitoring [16] and electrolyte concentrations in 60 mammary gland secretions [11] have also been used as complementary methods to predict 61 parturition. With advancements in image recognition technology, the use of continuous video-62 recording has also been investigated to monitor the onset of parturition [17]. The use of automated 63 64 mechanical and electronic equipment is being implemented in all aspects of bovine livestock production including feeding, rumination, estrus detection and calving prediction [18,19, 20]. Many 65 new devices to predict parturition are now available on the market: accelerometers to detect tail 66 67 raising and behavioral changes; abdominal belts to measure uterine contractions; intravaginal thermometers to evaluate temperature changes; and devices fixed in the vagina or at the vulvar lips 68 to detect calf expulsion [9]. 69

Cattle show a decrease in body temperature (Tb) before calving [21- 26]. The Tb in beef cattle 70 decreases from 48 to 8 h before calving and is not affected by the environmental temperature [27]. 71 Lammoglia et al. [27] suggested that the decrease in maternal Tb (eg. vaginal temperature) before 72 parturition is the consequence of the increased placental blood flow in the immediate precalving 73 period. This increased blood flow causes a raise in the foetal temperature, which may mitigate heat 74 75 loss by the neonatal calf in its new life ex utero. Interestingly, there is significant diurnal variation in the rectal and vaginal temperature of up to 0.5 °C, which is lower in the morning (0.2-0.3 °C) and 76 higher in the late afternoon (0.4-0.5°C), showing the importance of at least two repeated 77 measurements per day [25, 28]. Recently, the use of repeated Tb measurements (either rectal or 78 intravaginal) has become one of the most used tools of telemetry in livestock production due to its 79 80 easy assessment at the field level [28]. Variations in Tb has been used to predict the time of calving with different results [30, 31, 25]. Furthermore, there are no publications about the possibility of
using a particular temperature cut-off to predict parturition.

"Double muscling" is a heritable condition present in the Piedmontese cattle, that results in 83 muscular hypertrophy and hyperplasia [32]. The condition contributes to a low-fat and tender meat 84 that has made the breed well known and spread all over the world, as confirmed by the National 85 Association of Piedmontese Cattle Breeders (A.N.A.B.O.R.A.P.I.). Unfortunately, Piedmontese 86 cows and in general double-muscle breeds are more susceptible to difficulties at parturition [33, 1]. 87 Due to the increased trauma associated with parturition in double muscle beef cattle, there is an 88 increased need for accurate detection of the onset of parturition in order to provide assistance during 89 labor. Thus, the primary objective of this experiment was to establish an intravaginal temperature 90 91 (IVT) cut-off to predict calving in Piedmontese cows. Moreover, we aimed to clarify the utility of using an IVT as an automated method of calving detection in livestock farming to assist parturition 92 in the case of dystocia. 93

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#### 2- Materials and Methods

A total of 248 (219 multiparous and 29 primiparous) healthy pregnant Piedmontese cows from four 95 small-to-medium size herds (30 to 200 cows) located in the Piedmont region of Italy were enrolled 96 97 in the present study from November 2013 to April 2015. All cows were submitted to AI after heat detection and checked for pregnancy at 32-35 days post-AI by a skilled veterinarian via ultrasound 98 examination. All animals were housed in free stall barns throughout the entire year. Adequate ad 99 libitum feed was provided (hay, bent grass, and corn flour and soya) enriched with vitamins (A and 100 E) and mineral supplementation (Ca, P and Mg). All herds were officially free of IBR, tuberculosis, 101 102 brucellosis and enzootic bovine leucosis, and vaccinated against BVD and pneumonia.

The intravaginal electronic device (Medria Vel'Phone®, Châteaubourg, France) is an on-farm tool designed to predict (and alarm) calving by the IVT variation and expulsion of the device just before parturition. The device consists of a rigid tube with a plastic mandrel in which a temperature detector (TDetector) is inserted, covered with a plastic shell with ridges (Figure 1a). The tool is produced in two different sizes: large (for cows) and small (for heifers) (Figure 1b). Briefly, after

cleansing the device and the perineal area of the cow with a povidone-iodine solution (Betadine®), 108 the tubular support was gently inserted in the vulva and the TDetector was deposited deep into the 109 vagina (Figure 2). As indicated by the manufacturer, the TDetector should be introduced 7 d before 110 expected parturition, considered to be between 278 to 283 days in pregnancy depending on breed, 111 age, weight of the calf, milk yield, and season of parturition [34,35]. Once the TDetector is in the 112 cranial vagina, its thermometric sensor generates a radio wave signal which is transmitted to a 113 receiver (Figure 3) that analyzes the data and sends a text message via Global System for Mobile 114 (GSM) communication technology to the herd staff mobile phone; providing an alert of the 115 prediction (IVT variation) or the imminent beginning of calving (device expulsion, EXP). 116 Vel'phone monitors the IVT every 12 h (8:00 and 20:00), starting from the day of the device 117 118 insertion until delivery. A brief description of the Vel'Phone function process is described in Figure 4. 119

Although the literature has discordant definitions of dystocia [33, 36] for this study we categorizedparturitions into three groups:

*eutocic:* the autonomous and spontaneous calving. There is not an active intervention from
the staff members.

124 Dystocic parturitions were separated into two categories:

*assisted:* calving in need of assistance with a weak or a strong traction from the farmer, or
 requires intervention from a veterinarian.

127 - *caesarean:* parturition that requires a caesarean section.

128 *Statistical analysis* 

The day of TDetector insertion, IVT for each day (2 times per day), and date and temperature at EXP were exported from the Medria Vel'phone<sup>®</sup> to a Microsoft Excel (Microsoft Corporation, Redmond, WA) spreadsheet file. All data were statistically analyzed via R version 3.3.0 (R Core Team, Vienna, Austria). Descriptive statistics (mean, median, quartiles and standard deviation) were calculated using the summary functions of R software (*stats* package). Verification of the assumption of normality was evaluated using Shapiro-Wilk's test and F-Folded or Levene's 135 variance uniformity for all continuous data (*stats* package). The statistical significance level was set 136 at P < 0.05.

A scatter plot with a smooth curve fitted by Loess (princurve package) of all IVT measurements for 137 each cow by day was created based on measurements recorded from 60 h before parturition until 138 EXP. One-way ANOVA (car package) was used to assess temperature differences at 0, 12, 24, 36, 139 48 and 60 h before parturition (parturition = EXP). Next, the optimal IVT cut-off point to predict 140 calving (within 24 h), with the greatest summation of sensitivity (Se) and specificity (Sp) and its 141 respective positive predictive value (PPV) and negative predictive value (NPV), was calculated. For 142 this, two receiver operator characteristics (ROC) curves (pROC package) and the area under the 143 curve (cvAUC package) were constructed. For the first ROC curve, the average IVT were sorted 144 145 into three groups, 0-12, 24-36 and 48-60 h before parturition, and used as the predictor variable. The classifier (calved/not calved) was set as the calving result in the average IVT at 0-12 h (calved), 146 24-36 h (not calved) and 48-60 h (not calved) before parturition. A second ROC curve was created 147 in order to find the cut-off point which indicates the IVT drop difference with the greatest 148 sensitivity and specificity. For this ROC curve, the average IVT difference between 0-12 h with 24-149 36 h before calving (predictor = calved) and the IVT difference between 24-36 and 48-60 h before 150 calving (predictor = not calved) was used as the classifier. 151

After establishing the IVT cut-off point, factors associated with the drop in the IVT before 152 parturition were investigated. For this, binomial mixed effect models (package lme4, function 153 glmer) were constructed. The average IVT grouped at 0-12, 24-36 and 48-60 h before parturition 154 were dichotomised as: below the cut-off point as a positive predictor for calving, and above the cut-155 156 off point as a negative predictor for calving. The fixed effects tested were ease of calving (eutocia, traction, caesarean), lactation number (primiparous, multiparous), gestation length (continuous 157 variable), and season of calving (winter, spring, summer, fall). Moreover, the time (hours before the 158 expulsion of the device) where the IVT before calving was measured was also included as fixed 159 effect (IVT grouped at 0-12, 24-36 and 48-60 h before calving). All effects with P-values <0.2 160 (univariate) and their interactions were included in the final model, which was computed by 161

backward stepwise elimination. Due to its better fit in the model (lowest Akaike and Bayesian
information values), cow nested within farm was used as random effect. Results of the mixed effect
models are expressed as odds ratio (OR) with their respective confidence intervals (CI).

### 165 **3- Results**

Initially, 248 Piedmontese cows were included in the study. Thirty-seven cows were excluded due 166 to unavailability of data, or because the device remained in the vagina for less than three days 167 before its expulsion. Therefore, for the final data analysis, a total of 211 cows were included (27 168 primiparous and 184 multiparous). Mean gestation length was 291.5  $\pm$  13.7. (primiparous, 292  $\pm$ 169 14.1 days and multiparous,  $289 \pm 9.2$ , days). The TDetector was kept in the vagina an average of 170  $8.5 \pm 6$  d. A total of 52.6% (111/211) of the subjects showed dystocia (assisted or caesarean); 171 172 primiparous cows showed a higher tendency for dystocic calvings in comparison to multiparous cows (70.3% (19/27) vs 50.0% (92/184), P = 0.06). Although no statistical significance was found, 173 primiparous cows had more caesarians and assisted deliveries in comparison with multiparous (25.6 174 175 % vs 23.9%, P>0.05).

The average IVT trend from 60 h before parturition until EXP, is shown by the Loess curve (Figure 176 5). ANOVA was applied to compare the average IVT between different time periods, from 0 to 60h 177 before calving (Table 1). A significant decrease in the IVT was noticeable from 24 h before until 178 EXP. The mean IVT was grouped at 0-12, 24-36 and 48-60 h before parturition (Table 2), and a 179 significant drop was recorded from 24-36 h to 0-12 h ( $38.48 \pm 0.25$  vs  $38.12 \pm 0.25$  °C). The ROC 180 and AUC outputs used for defining the cut-off of IVT to predict parturition 24 h before the calving 181 event are illustrated in Figure 6. Based on this analysis, the IVT cut-off to predict calving was set at 182 183  $38.2^{\circ}C$  (Se = 86%, Sp = 91%; PPV = 80%, NPV= 88%; AUC = 0.89) (Table 3). When a variation of IVT was considered as the value to predict parturition, the cut-off was -0.21°C (indicating a drop 184 in IVT) with Se = 66%, Sp = 76%, PPV = 67%, and NPV = 69% (AUC = 0.72) (Table 3). Results 185 of the binomial mixed effect models revealing the factors associated with a variation in the IVT 186 before calving are illustrated in Table 4 and indicate that parity, type of calving, season, and length 187 of gestation did not influence the change in intravaginal temperature before parturition. 188

## 189 **4- Discussion**

Although different IVT devices have been used in research for years, very few are present in the 190 market for its use on commercial farms [9] and most of them lack evidence about the degree of 191 effectiveness to predict parturition. This study is one of the first to use an intravaginal sensor system 192 available on the market to evaluate the accuracy of IVT as calving predictor on commercial farms. 193 In this system, IVT measurements were immediately and wirelessly recorded with a smartphone, 194 allowing for better control and notification at the time of delivery than retrospective data collection 195 (useful for research but not for commercial use). The TDetector stayed in the vagina for more than 196 one week (in some cases up to 20 d), and did not cause any secondary effects or lesions in the cow 197 (data not shown). Eventually, a temporary, mild vaginitis was observed. Piedmontese cattle have a 198 199 gestation period which is slightly longer than other beef cattle [37], which was also demonstrated in our study by the average gestation length of 292.5 d. Thus, in agreement with the Piedmontese 200 national association, Piedmontese cattle calved hyper mature or oversized newborn calves [39]. 201

202 The physiological variability of the day of delivery makes it hard to foresee parturition, resulting in the threat of unassisted dystocia. For this reason, different ways to evaluate and anticipate the 203 moment of parturition have been studied for years, including the development of various 204 205 technologies [9]. Body temperature and IVT decreases have already been described in the scientific literature [25, 28, 22, 23, 27 24, 40]. Temperatures measured continuously by data logging 206 thermometers were on average 0.5°C lower at parturition than when measured 48 h before delivery, 207 in both dairy and beef cows [25,28]. Furthermore, when sensors were used to evaluate the drop of 208 IVT as the delivery predictor, better accuracy for calving prediction within 24 h before parturition 209 210 was reported [25, 28, 9]. In beef cows, Aoki and collaborators [25] found that the probability of calving within 36 h by continuous (every minute) sampling to detect a variation > 0.3 °C in IVT 211 during seven days prepartum was over 83%. In our results, IVT starts to decrease from 60 h before 212 213 parturition, but a significant reduction in temperature was seen only 24 h before parturition.

Intravaginal and body temperatures may vary due to several extrinsic or intrinsic factors (i.e. environmental temperature, degree of physical exercise, and the metabolic state of the animal)

[25,28]. However, according to Lammoglia et al. [28] during the immediate precalving period (48 216 to 8 h before parturition) Tb is not influenced by the environmental temperature nor sex of the calf. 217 In our study, physical exercise is minimal before parturition because they are confined in a calving 218 pen. All cows were housed in farms from the same geographical area with similar management and 219 furthermore were in the same metabolic state (just before delivery), therefore, we did not consider 220 these variables in the statistical model used in this study. When season and length of pregnancy 221 effects were evaluated, no effect on the change in intravaginal temperature before parturition was 222 found. 223

Because a significant diurnal variation in the rectal and vaginal temperature has been reported [25, 28], in this study the intravaginal temperature was measured 2 times per day at 8 am and 8 pm. Consequently, the diurnal variation in temperature will not influence estimates of the change in temperature that occurs before parturition. Moreover, we indirectly demonstrate this with ROC curves which showed a relatively high Se and Sp.

229 When we used the ROC curves to calculate the best cut-off for IVT variation as the delivery predictor, a decrease of 0.21 °C was found to be the change in temperature with the highest Se and 230 Sp. Similarly, Burfeind et al. [28] investigated IVT decreases as a calving predictor in dairy cows 231 using a controlled internal drug release tool modified to operate as a simple thermometer in the 232 vaginal cavity  $6 \pm 2$  d prepartum. Burfeind et al. [28] found that a 0.2-0.3°C temperature decrease 233 on the day of calving compared to 24 h before parturition, showed a higher Se (76%) but a similar 234 Sp (79%) in comparison to our results. Therefore, in this aspect a low accuracy is obtained with a 235 decrease of 0.2°C of IVT. Consequently, IVT variation may not be the ideal measurement to predict 236 237 parturition in dairy or beef cattle. Because of the lack of accuracy in the trend of the IVT during the last 24 h as predictor parameter for parturition, we aimed to use the average IVT itself as a cut-off 238 value to predict parturition within 24 h. The ROC curve for the IVT cut-off within 24 h prepartum 239 showed that when the average IVT dropped to 38.2°C or below, parturition can be predicted with 240 higher AUC and increased Se and Sp in comparison to a change in IVT during the last 24 h 241 prepartum. It is important to mention that no other studies have considered an average IVT as the 242

cut-off to predict calving; this novel approach appears to be more accurate than checking for achange in IVT.

As previously discussed, dystocia can have a large economic impact on farms due to calf death, 245 veterinary costs, decreased rebreeding efficiency, and injury or death of the cow. Cow-calf Health 246 and Productivity Audit (CHAPA) studies indicate that dystocia is responsible for 33% of all calf 247 losses and 15.4% of beef cattle breeding losses. Anderson et al. [41] reported that 57% and 67% of 248 calf losses in two studies conducted in Minnesota and Michigan were from dystocia. Our study 249 showed Piedmontese cows have a similar percentage of dystocia (53%). Interestingly, not all the 250 cows that underwent dystocia showed any calf losses at parturition (due to the prompt assistance). 251 Although the genetic selection in the last decades has worked to improve easier calvings and 252 253 smaller calf dimension, dystocic parturitions and mainly caesarians are still a problem in the reproductive management of Piedmontese cows; although in a lower percentage than in other 254 double muscle breeds such as Belgian Blue [42]. Nevertheless, a precise and accurate prediction of 255 parturition can improve availability of calving assistance: preventing newborn calf losses and 256 improving future reproductive performance of the cow. 257

258 **5-** Conclusions

A simple commercial device to predict calving is a useful tool to improve management of parturition in modern beef farms. In this regard, the average IVT seems to be a better parameter to predict the parturition within 24 h than the drop in IVT. In farms where the percentage of dystocia is high, an accurate prediction of parturition can significantly improve farmers ability to assist cows at the time of delivery.

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368	<b>Figure 1.</b> Medria VelPhone <sup>®</sup> consist of a rigid tubular tube with a plastic mandrel in which is		
369	inserted a temperature detector (TDetector) covered by a plastic shell with ridges (A). The tool is		
370	pro	duced in two different sizes: large for cows (right) and small for heifers (left) (B).	
371	Fig	ure 2. Medria VelPhone® generates a radio wave signal which is transmitted to a receiver that	
372	analyses the data and sends a text message via GSM technology to the herd staff mobile phone,		
373	providing an alert of the prediction or the imminent beginning of calving.		
374	Fig	ure 3. First, the TDetector device was plunged into a bucked filled with an antiseptic	
375	solution (A) and inserted in the tubular support (B). Before the introduction of the device into		
376	the	vagina, the perineal region of the cow was cleansed with Betadine® (C). Next, the tubular	

support was gently inserted in the vulva (D) and the TDetector was deposited deep into thevagina (E).

**Figure 4.** Functional process of the Vel'Phone. At Phase 1 the TDetector is inserted into the vagina seven days before the expected parturition. Next, the Vel'Phone device starts to measure the intravaginal temperature (IVT) every 12 h (Phase 2), and Phase 3 starts when a variation in the IVT occurs 24 h prior parturition. Phase 4 consist of the expulsion of the Vel'Phone, and Phase 5 is the imminent calving. An SMS is sent to the farmer's cellphone at Phase 3 (IVT variation) and at Phase 4 (expulsion of the device).

Figure 5. Scatterplot with Loess curve of the average intravaginal temperature from day 30 beforeto the day of parturition.

Figure 6. Receiver operating characteristic curves showing the vaginal temperature drop (A) and
(B) the average vaginal temperature cut-off points to predict calving within 24 h in Piedmontese
cows (crosshair marks highest point for sensitivity and specificity).

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