

# Application of Behavioural Biometrics and Biomarkers for Calving Prediction in Bovines

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## ABSTRACT

Timely and accurate prediction of parturition in bovine is crucial for optimizing maternal and fetal health, preventing dystocia and minimizing periparturient complications. A multi-modal approach that integrates behavioural, physiological and molecular indicators enables more precise calving prediction. Behavioural cues, such as increased frequency of lying and tail-raising in the final six hours before parturition, combined with pelvic ligament relaxation triggered by elevated estrogen and relaxin, offer reliable external signs of impending parturition. Key physiological markers include a significant drop in body temperature, vaginal temperature and ruminoreticular temperature, alongside hormonal shifts particularly a decrease in progesterone and increases in prostaglandin F2 $\alpha$  and estradiol (E2), all of which are having significance in predicting parturition. Advanced monitoring systems and automated sensors capable of tracking these parameters have proven valuable in improving periparturient cow management. Biomarkers, such as increased levels of metalloproteinases, lactate dehydrogenase,  $\alpha$ 1-acid glycoprotein and haptoglobin, combined with a decrease in transthyretin, further signal the onset of parturition. In addition, distinct metabolite profiles including changes in prostaglandins and lipid metabolites such as glycerophospholipids, lysophosphatidylcholines, and sphingolipids, provide molecular insights into the proximity of parturition. This comprehensive approach, combining behavioural, endocrine and biochemical markers, enhances the accuracy of parturition prediction, ultimately improving reproductive success and reducing reproductive losses in cattle.

**Keywords:** Behaviour, Biomarker, Bovine, Hormones, Parturition, Prediction

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## INTRODUCTION

Parturition, or the process of giving birth, represents a critical event in livestock management. This process begins with the activation of the fetal hypothalamus-pituitary-adrenal (HPA) axis, which plays a pivotal role in initiating labor. Near parturition, fetal cortisol triggers an increase in estradiol production, facilitating the relaxation of the birth canal and activating cervical secretory functions. Prostaglandin F2  $\alpha$  (PGF2 $\alpha$ ) initiates smooth muscle contractions and induces regression of the corpus luteum, subsequently reducing progesterone levels. The combined elevation of estradiol and PGF2 $\alpha$  concentrations intensifies uterine contractions. In conjunction with estrogen, relaxin contributes to pelvic ligament relaxation, notably the sacrosciatic ligament (Senger, 2003; Streyl *et al.*, 2011). Additionally, oxytocin surges, and these hormonal shifts lead to the depolarization of myometrial cells, thereby enhancing uterine motility, ultimately resulting in fetal delivery (Wood, 1999).

Around the time of calving, dairy cows are at an increased risk of calving associated and postpartum health complications. Safe and timely parturition is essential for reproductive success in cattle, as premature labor can lead to preterm birth and neonatal mortality, while delayed parturition may cause fetal distress, in-utero death, or dystocia. Accurate prediction of parturition is crucial to optimize animal care and reduce the risk of complications, including dystocia (difficult calving), vaginal lacerations, retained fetal membranes, and subsequent clinical metritis or endometritis, which can adversely impact both maternal

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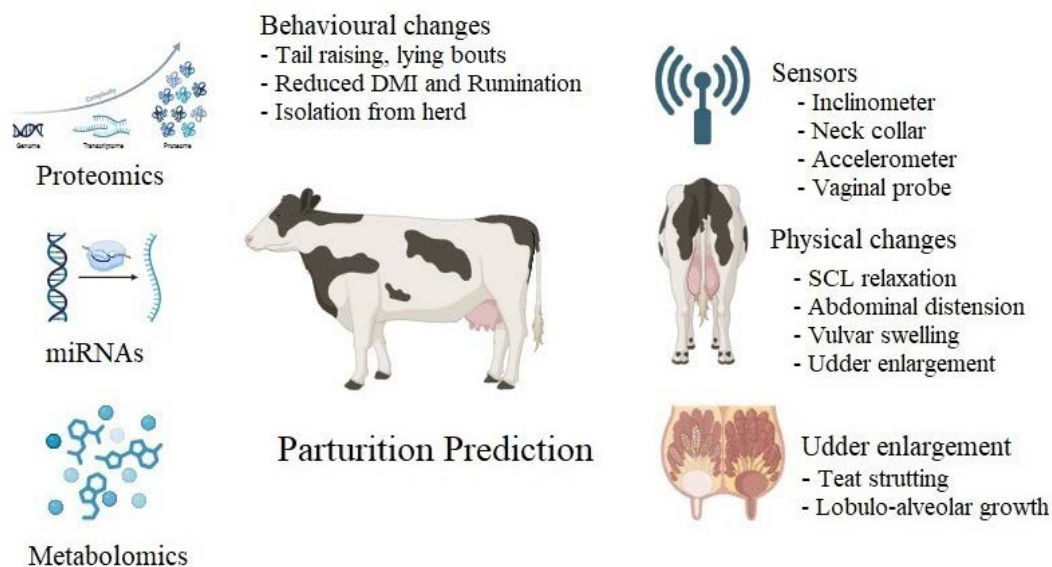
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and calf health. Reproductive losses can compromise fertility, reduce milk production, impair future performance, and result in significant economic losses within cattle production. Thus, accurate parturition prediction is essential to prevent reproductive losses, facilitate planning for elective Cesarean sections, and determine the appropriate time to move a cow to the calving pen (preferably few days before calving). Predicting parturition also enables the planning of human supervision, especially during nighttime, and enhances overall management practices and monitoring, ultimately improving maternal and neonatal calf health outcomes (Saint-Dizier and Chastant-Maillard, 2015). To predict parturition accurately, it is essential to understand and recognize the hormonal, biochemical, metabolic, physical, and behavioural changes associated with calving (Fig.1).



**Fig. 1:** Methods and strategies for predicting parturition in bovines

## PHYSICAL, BEHAVIOURAL AND TEMPERATURE CHANGES ASSOCIATED WITH PARTURITION

### **Physical Changes during Pre- and Peri-Parturient Stages**

Physical changes, such as the relaxation of the sacrospinal ligament, are considered reliable indicators for predicting calving within 12 to 24 hours (Kornmatitsuk *et al.*, 2000; Hulsen, 2006). This relaxation manifests as a noticeable indentation beside the tail head (Dyce *et al.*, 2010). In fact, the relaxation of pelvic ligaments serves as one of the most reliable clinical indicators when assessed alongside other parameters (Streyl *et al.*, 2011). Other observable changes, including abdominal distension and udder engorgement (often referred to as “bagging up”), vulvar swelling, and teat strutting, are common signs of impending parturition, however, they are less reliable indicators for precise prediction of parturition timing (Hofmann *et al.*, 2006).

### **Behavioural Changes Associated with Parturition**

Behavioural changes preceding parturition include reduced feed intake, decreased rumination time, increased activity, frequent lying bouts, tail raising, and ground-licking (Miedema *et al.*, 2011). During parturition, discomfort caused by regular myometrial contractions leads to restless behaviours such as frequent turning and looking around, vocalizations, licking and pawing at bedding material, lifting and waving the tail, increased mobility, separation from the herd, frequent changes between lying and standing positions, and interrupted feeding patterns (Phillips, 2002; Miedema *et al.*, 2011; Nagel *et al.*, 2020).

Dry matter intake (DMI) has been observed to decrease by approximately 30% on the day of calving compared to the previous day (Huzzey *et al.*, 2007; Proudfoot *et al.*, 2009; Schirmann *et al.*, 2013). Additionally, rumination time declines progressively, starting about one week before calving, with a sharp decrease noted from 24 hours to 3 hours before calving compared to the dry period (Calamari *et al.*, 2014; Pahl *et al.*, 2014; Ouellet *et al.*, 2016; Borchers *et al.*, 2017). This gradual reduction in DMI and rumination time is attributed to the expanding gravid uterine volume, which limits digestive capacity, and the stress associated with the calving process (Hulsen, 2006; Miedema *et al.*, 2011).

Notably, increased lying bouts and frequent tail-raising, observed 24 hours prior to parturition, are among the most accurate indicators of impending calving, as documented through video observations (Miedema *et al.*, 2011). A decrease in total lying time and an increase in lying bouts have also been reported 24 hours before calving (Black and Krawczel, 2016; Rice *et al.*, 2017; Sepúlveda-Varas *et al.*, 2018; Hendriks *et al.*, 2019; Miedema *et al.*, 2011). Tail-raising, a long-established indicator of calving, becomes markedly more frequent approximately 6 hours before calving (Miedema *et al.*, 2011; Barrier *et al.*, 2012; Jensen, 2012).

### **Body Temperature Changes**

Body temperature in cattle typically decreases shortly before parturition. Rectal temperatures have been observed to drop by approximately 0.5 to 1.6 °F the day before calving, effectively predicting parturition within a day (Streyl *et al.*, 2011). A marked decline in vaginal temperature, ranging from  $\geq 0.3$  to  $\geq 0.5$  °C, often occurs within 24 to 48 hours prior to calving (Aoki *et al.*, 2005; Burfeind *et al.*, 2011; Ricci *et*

*al.*, 2018). Similarly, ruminal temperatures, measured using ruminal boluses, decrease by about 0.3 °C (0.54 °F) around 24 hours before parturition (Cooper-Prado *et al.*, 2011). It has been suggested that the estrogen-to-progesterone ratio is a primary factor regulating body temperature in the prepartum period (Rexha *et al.*, 1993).

Recent studies on Murrah buffaloes demonstrated that eye temperature and udder skin surface temperature (USST), as measured by infrared thermography, also showed significant declines 6-12 hours before calving, coinciding with a decrease in progesterone concentration. However, the diagnostic sensitivity of eye temperature and USST ranged from 50% to 57% at 48 hours pre-calving, indicating limited predictive value for calving in buffaloes (Teja *et al.*, 2023).

## SENSORS AND DEVICES TO PREDICT ONSET OF PARTURITION

In addition to traditional methods that assess external and behavioural signs, advanced technologies now offer more precise ways to detect the onset of calving. These systems include sensors that monitor behaviours such as rumination, eating, activity, and tail raising, providing real-time alerts of impending parturition.

Various sensors have been developed for calving prediction. For instance, inclinometer monitors tail-raising behaviour, which significantly increases in frequency within the final six hours before calving (Miedema *et al.*, 2011). However, they can be dislodged or damaged by licking, chewing, constant tail movement, or during fetal delivery, which may compromise the detection of behavioural cues. Neck collars are another sensor type that monitors feeding time, rumination, and resting behaviours, while accelerometers attached to the hind limbs track step count and lying bouts. Additionally, sensors are available to monitor core body temperature decreases in areas such as the vaginal canal, ventral tail base, ear surface, and reticulo-rumen (Saint-Dizier and Chastant-Maillard, 2015; Szenci, 2022).

Temperature monitoring at the ventral tail base, for example, has shown a consistent drop in core body temperature approximately 24 hours before calving. This decrease is due to reduced blood flow caused by fetal pressure on the tail blood vessels (Walls and Jacobson, 1970) and can be detected by sensors attached to the ventral tail base surface (Koyama *et al.*, 2018). Vaginal probes, placed within the vaginal canal or attached to the vulvar skin, detect the expulsion of the allantochorion and calf with high accuracy, though they pose a mild risk of vaginal laceration or inflammation upon insertion (Ricci *et al.*, 2018; Saint-Dizier and Chastant-Maillard, 2015; Szenci, 2022).

Furthermore, researchers have developed machine learning algorithms that predict calving within 1-5 days by analyzing activity, temperature, and drinking behaviours using reticuloruminal boluses, which measure both activity and temperature. These algorithms achieve a sensitivity of

75.84% and specificity of 92.99% (Vázquez-Diosdado *et al.*, 2023). The combination of multiple sensors can significantly enhance the accuracy of predicting the onset of parturition.

## BIOMARKERS IN PREDICTING PARTURITION

Parturition is driven by complex molecular interactions and the production and release of various bioactive mediators at the maternal-fetal interface, leading to uterine contractions, cervical changes, and ultimately fetal expulsion. A significant increase in fetal cortisol stimulates estrogen production and progesterone withdrawal, which is key to initiating parturition in cattle (Lye, 1996). This shift in placental steroid production upregulates contraction-associated proteins in the myometrium and releases uterotonic prostaglandins, driving uterine contraction and fetal expulsion (Gyomory *et al.*, 2000; Whittle *et al.*, 2000; Shenavai *et al.*, 2012). Additionally, estrogen-induced increases in connexin-43 expression and myometrial transcription factors (*c-fos*, *c-myc*, *c-Jun*) promote the synthesis and release of stimulatory uterotonins like prostaglandins and oxytocin (Lye, 1996). About 36-48 hours before birth, the metabolite 15-keto-13,14-dihydro PGF<sub>2</sub>α (PGFM) also begins to increase, signaling impending parturition (Shenavai *et al.*, 2012).

### Endocrine Biomarkers

The most accurate prepartum hormonal change is a decrease in plasma progesterone (P<sub>4</sub>), where concentrations below 1.2 ng/mL indicate calving within 12-24 hours (Strey *et al.*, 2011; Hiew *et al.*, 2020; Monteiro *et al.*, 2024). While estradiol (E<sub>2</sub>) levels rise one day before parturition (~1 ng/mL) and drop significantly within a day postpartum (~0.4 ng/mL), its variability makes it less reliable, with a high rate of false prediction (Saint-Dizier and Chastant-Maillard, 2015; Monteiro *et al.*, 2024). Cortisol, prolactin, and PSP-B also increase before parturition but are considered less accurate as markers for calving onset (Yi *et al.*, 2022; Hiew *et al.*, 2020; Monteiro *et al.*, 2024). Blood glucose levels increase on the day of parturition, likely in response to hypercortisolemia; a glucose concentration above 79 mg/dL offers a practical, low-cost way to predict parturition within 6-12 hours (Hiew *et al.*, 2020).

### Proteomic Biomarkers

Changes in plasma protein expression around calving may serve as critical markers to regulate parturition in dairy cows. Quantitative proteomics play a pivotal role in identifying these biomarkers and unraveling the molecular pathways of parturition (Boylan *et al.*, 2010; Kolla *et al.*, 2010). Acute-phase proteins, such as haptoglobin and α<sub>1</sub>-acid glycoprotein (AGP), rise significantly near parturition, reflecting stress and inflammatory responses (Bionaz *et al.*, 2007; Bossaert *et al.*, 2012). Mannose-binding protein C (MBL) is essential for successful pregnancy, with lower levels associated with premature birth and reduced birth weight, highlighting its potential as a biomarker (Bouwman *et al.*, 2006). Additionally,

proteins like metalloproteinase inhibitors and lactate dehydrogenase (LDH) are integral to tissue remodeling, cervical ripening, uterine contractions, and placental separation, indicating their role as parturition markers (Wawrzykowski *et al.*, 2018). Elevated serum LDH levels also correlate with pregnancy complications, signaling potential maternal and perinatal risks (Afroz *et al.*, 2015).

Steroid hormones are primarily bound to transport proteins such as corticosteroid-binding globulin (CBG), which regulates steroid bioavailability to target tissues (Fernández-Real *et al.*, 1999). During parturition, a decrease in CBG levels increases free cortisol, promoting gluconeogenesis and raising glucose levels essential for parturient energy demands (Berlusconi *et al.*, 1995; Risberg *et al.*, 2016; Bae and Kratzsch, 2015). Despite these findings, the ontogeny and glycoform composition of CBG remain insufficiently characterized. Further proteomic studies are warranted to validate these biomarkers and deepen our understanding their role in bovine parturition mechanisms and explore their possibility for calving prediction.

### Metabolomic Markers

The periparturient period in dairy cows is marked by significant endocrine and metabolic shifts. Targeted metabolomics quantifies specific metabolites in crucial metabolic pathways, while untargeted metabolomics compares metabolite levels between control and experimental groups, aiding in disease marker screening (Patti *et al.*, 2012; Li *et al.*, 2017). It has been observed that glycerophospholipid and sphingolipid levels decrease approximately 10 days before and 3 days after calving, while lactate concentrations, as TCA cycle precursors, increase near parturition, particularly on the day of calving (Luo *et al.*, 2019; Monteiro *et al.*, 2024). Future studies should employ targeted metabolomics to verify metabolites and regulatory enzymes in these pathways.

### Circulating MicroRNAs (miRNAs)

MicroRNAs (miRNAs), approximately 17-24 nucleotide-long non-coding RNA molecules, regulate gene expression post-transcriptionally, playing a role in pregnancy maintenance (O'Brien *et al.*, 2018; Chatterjee and Thakur, 2024). Their high stability makes them potential non-invasive disease biomarkers (Mitchell *et al.*, 2008; Morales-Prieto *et al.*, 2013). In humans, miRNA profiling can be used for early pregnancy detection and assessing pregnancy complications (Mitchell *et al.*, 2008; Miura *et al.*, 2010). Although bovine research on miRNAs as parturition predictors is limited, this field presents a promising direction for developing alternative, non-invasive biomarker methods for parturition prediction. Further in-depth analyses are required to identify miRNAs and their target genes for use as reliable parturition markers in dairy cows.

## CONCLUSION

Predicting parturition in dairy cows is essential for managing parturition and reduce the calving associated complication. Traditional methods, though useful, are increasingly complemented by advanced sensor technologies that monitor behavioural and physiological indicators in real time. Molecular cues, such as specific hormones, proteins, and nucleic acids, offer promising biomarkers for calving prediction, though further validation is needed. By combining automated sensor data with genomic, proteomic, and metabolomic biomarkers, we can enhance calving prediction, improve dam and calf welfare, reproductive efficiency and overall performance of dairy herd.

## FUTURE DIRECTION

Advancing real-time sensor technology holds significant promise for improving the accuracy of calving predictions. Integrating automated sensor systems to monitor physiological and molecular cues could provide a more reliable, real-time approach for calving detection, enhancing both animal welfare and farm management. Future research should focus on discovering and validating genomic, proteomic, and metabolomic biomarkers to accurately predict parturition onset and identify potential calving associated complications. Further investigation into circulating nucleic acids (CNAs) and microRNAs (miRNAs) as biomarkers holds a promising area to explore their potential for calving prediction. Biomarkers could increase prediction accuracy and provide a practical, non-invasive approach for use in farm environments.

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