Morphokinetic features of the bovine embryo may be associated with lethal chondrodysplasia condition

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Abstract

This case report details the identification of lethal chondrodysplasia (LCD) in a Holstein embryo following embryo transfer and initial normal development during pregnancy. Lethal chondrodysplasia is a genetic disorder characterized by severe skeletal deformities during the developing pregnancy, leading to detrimental live-calf outcomes and cow-calf welfare. In this study, an in vitro produced (IVP) Holstein embryo was classified stage 7, quality grade 2, by an experienced bovine embryologist and was transferred to a recipient Holstein heifer. A video of this embryo was recorded with a View 4k camera attached to a Nikon SMZ 1270 microscope before loading and transferring. The recipient showed normal pregnancy progression, but resulted in abortion near ~180 days post embryo transfer. Post-abortion examination revealed severe skeletal deformities consistent with LCD. Upon analysis, embryo morphokinetics which evaluates developmental patterns and cellular activity from the embryo video showed normal activity levels, but higher variability and standard deviation in the LCD-affected embryo. This case underscores the limitations of initial embryo evaluations and highlights the potential of advanced embryo analysis technologies, such as computer vision morphokinetic assessment, to detect genetic abnormalities earlier and improve breeding programs' accuracy.

Key words: bovine lethal chondrodysplasia, bovine embryo transfer, embryo morphokinetics

Introduction

Lethal chondrodysplasia (LCD) is a well-documented genetic disorder in cattle caused by a recessive gene that profoundly influences skeletal development, leading to anomalous limb and craniofacial formations.[1] Particularly in Holsteins, this condition presents significant challenges due to its detrimental effects on calf survival and overall welfare.[1] The presence of LCD is rare and difficult to detect, as diagnosis of genetic mutations typically require genetic testing of a biopsied embryo or genetic testing of a bovine fetus through amniocentesis, both of which are not routine or commonly used procedures as they are not practical or affordable for use in the bovine veterinary industry. Although LCD is rare and difficult to detect, the increasing use of in vitro embryo production has opened new opportunities to study early signs of LCD and deepen our understanding of this genetic disorder.

The advent of in vitro embryo production and embryo transfer has accelerated genetic progress to improve herd health, productivity, animal welfare and production economics. Despite these advantages, there is concern that in vitro-produced (IVP) embryos can lead to adverse effects including lower conception rate, increased early embryonic loss, and higher incidence of genetic mutations.[2-6] Due to the challenges in detecting these genetic mutations, these genetic abnormalities tend to go undetected in most routine livestock embryo transfer scenarios. While rare, these genetically abnormal embryos can result in seemingly normal pregnancies and fail to be detected until the incidence of a late term abortion or parturition, which could result in animal welfare challenges and significant economic losses to the producer. However, meticulous record-keeping, documentation, and management practices during embryo evaluation, transfer, pregnancy and parturition can allow opportunities to study incidence of disorders, such as LCD, in ways that are not possible during natural breeding to potentially allow early detection of affected embryos to prevent losses associated with the disorder.

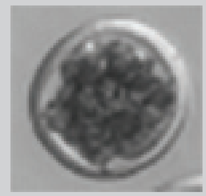
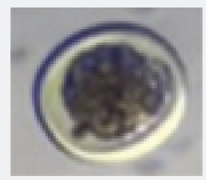
This case study recounts the identification of LCD in a Holstein embryo after successful embryo transfer, and initial phenotypically normal development, and serves as a poignant reminder of the imperative for rigorous embryo assessment to mitigate the occurrence of such disorders in breeding programs.

Case presentation

Ovum pick-up (OPU), an ultrasound guided process to aspirate follicular fluid from the ovaries of a donor female, was used to collect oocytes from a Holstein heifer at 312 days of age. Collected oocytes were fertilized with conventional semen from a Holstein bull and cultured in a commercial IVF laboratory. On day 6 of culture, embryos were shipped in Micro Q Transportable Incubator (Micro Q Technologies, Mesa, AZ, USA) to a commercial Holstein heifer breeding facility where all embryos were evaluated upon arrival on day 7.

Unbeknownst to the embryologist, one of the embryos was affected with LCD. After a morphological evaluation by an experienced bovine embryologist using a Nikon SMZ 1270 microscope, the embryo was classified as "stage 7", "grade 2" on day 7 post-fertilization in accordance with International Embryo Technology Society (IETS) standards. Embryo evaluation methods are described in detail by the IETS and aim to classify embryo development and quality with a series of codes. Pre-implantation embryo stage of development is represented by codes 1-9, in which 1 represents an unfertilized oocyte, the lowest stage of development, and 9 represents an expanded hatched blastocyst, which is an embryo that consists of differentiated cell types that has undergone expansion, hatched from the zona pellucida, and is preparing to implant into the endometrium (Table 1). Embryo quality grade is represented by codes 1-4, in which quality grade 1 represents an "excellent or good embryo" that is well-defined, spherical in shape with no visible defects. This is considered the highest quality

Table 1: Stage of Development. Description of codes associated with embryo stage of development with associated images.

Code	Description	Image
1	Unfertilized oocyte	
2	2- to 12-cell	
3	Early morula	
4	Morula	
5	Early blastocyst	
6	Blastocyst	
7	Expanded blastocyst	
8	Hatched blastocyst	
9	Expanded hatched blastocyst	

and most likely to lead to successful pregnancy. Quality grade 4 is a dead or degenerating embryo that is poorly developed with major fragmentation, uneven cell size and visible defects which are known to have a low chance of successful implantation or pregnancy and are generally not recommended for transfer (Table 2).

Based on this examination, the embryo was deemed a stage 7 expanded blastocyst suitable for transfer, though the embryologist documented an unusual protrusion of the zona pellucida and trophectoderm, which was regarded as insignificant and did not hinder eligibility for embryo transfer (Figure 1). This embryo was one of the 89 embryos selected for transfer into eligible recipient heifers that day.

Prior to transfer, a 30-second video was recorded for all embryos available that day, including the affected embryo. Embryo videos were recorded on a View4K camera at 90x magnification with a Nikon SMZ 1270 microscope mounted to the trinocular port of the microscope. While imaging equipment of embryologists vary based on personal preferences, this equipment is consistent with livestock embryologists and embryo transfer practitioners who work with fresh embryos.

Up to 6 embryos were recorded in each video, with the embryos oriented with at least 10 um distance of separation. The environmental conditions during evaluation showed an embryo temperature of 32.8 °C, with an ambient environmental temperature of 6.3 °C. All additional selected embryos, including the affected embryo, were transferred into eligible recipient females as determined by estrous cycle, presence of a corpus luteum and digital evaluation of uterine tone. Routine pregnancy checks were performed by a licensed veterinarian via ultrasound at 35-, 60- and 180-days post-transfer. It was documented that the recipient carrying this affected embryo initially exhibited excessive fluid accumulation at day 35 post-transfer, which was palpable through transrectal examination. However, subsequent checks at days 60 and 180 showed normal pregnancy progression.

Unexpectedly, beyond day 180, the recipient aborted the pregnancy. Examination of the aborted fetus revealed severe skeletal deformities consistent with LCD, confirming the diagnosis.

Embryo and pregnancy outcomes

Of the 89 embryos transferred, 40 (44.9%) resulted in pregnancy by day 35, including one LCD-affected embryo. Of the 89 embryos, 49 (55%) shared the same sire. As it is expected LCD consists of genetic origins, these 49 embryos were used in the following analysis. Of the 49 embryos transferred on this date, 24/49 (48.9%) embryos produced pregnancies (one being the LCD-affected embryo). The LCD-affected embryo was the only embryo which resulted in abortion.

To determine if the presence of the genetic defect is observable in the embryo video data, all videos of the 89 embryos were processed with graphic imaging processing techniques using EmGenisys software (EmGenisys, TX, USA). In this process, video data is processed to reveal embryo change, or

Table 2: Quality Grade. Description of codes associated with embryo quality grade. Embryo images depict stage 4 morulas of varying quality grades.

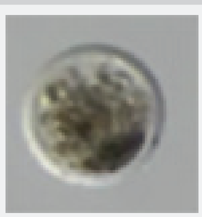
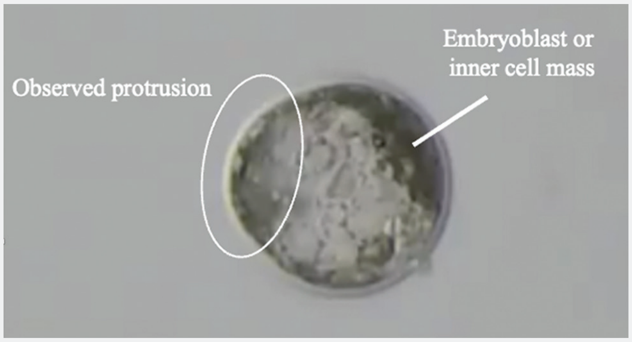
Code	Description	Image	Traits and characteristics
1	Excellent or good		The embryo is well-defined, spherical in shape with no visible defects
2	Fair		The embryo shows minor irregularities in shape or cell division, extruded cells, or fragmentation
3	Poor		The embryo shows irregularities and fragmentation that consume greater than 50% of the embryo's volume
4	Dead or degenerating		The embryo is poorly developed with major fragmentation, uneven cell size and visible defects

Figure 1: The lethal chondrodysplasia-affected embryo. Embryo was classified as a stage 7, grade 2 blastocyst according to International Embryo Technology Society standards. The embryo appears to be an expanded blastocyst and contains a clearly defined embryoblast and trophectoderm. The embryo is not perfectly spherical in shape, as evidenced by the protrusion opposite of the embryoblast.



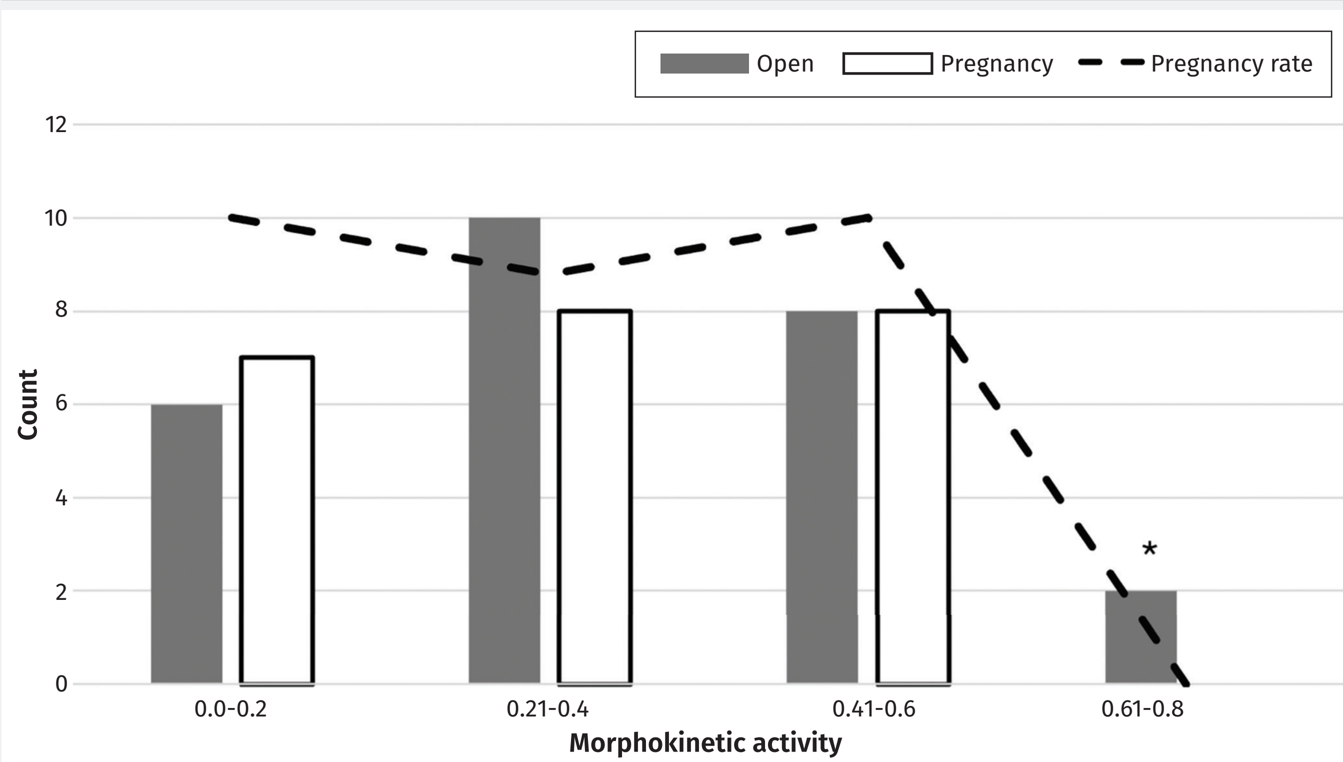
morphokinetic activity frame-by-frame. Details of this technique are published by Wells et al., 2022.[7] In this analysis, embryo morphokinetic change was quantified 3 times per second, or every 20 frames, given that the videos were recorded at a rate of 60 frames per second, resulting in a total of 90 calculations per embryo. The mean morphokinetic activity and standard deviation were then calculated for each embryo.

To better control for genetic variation, a separate analysis was conducted using the 49 embryos that shared a sire, as these embryos are genetically more similar to each other than to the unrelated embryos. This allowed for more precise identification of any unique patterns associated with the LCD-affected embryo. A subsequent, broader analysis was then performed on the full set of 89 embryos to assess whether the observed findings were consistent across all embryos.

Mean morphokinetic embryo activity of the related embryos ranged from 0.2-0.7. These embryos were sorted into 4 groups based on mean morphokinetic activity level and 35-day pregnancy outcomes were compared (Figure 2). Most pregnancy producing embryos demonstrated lower mean morphokinetic activity values, as consistent with other studies (Figure 2).[8]

Mean embryo morphokinetic activity of the LCD embryo was 0.28, which is consistent with other embryos that produced pregnancies. Of the 14 embryos in the morphokinetic activity level 0.21-0.4 group, 8/18 (44.5%) produced pregnancies (Figure 2). While the mean morphokinetic activity of the LCD-affected embryo appeared normal, the frame-by-frame activity was plotted and showed the LCD embryos activity exhibited more variation and variable activity patterns than the other embryos in this morphokinetic activity level group. Higher variability in morphokinetic activity may indicate developmental instability, which could be linked to early embryonic loss or genetic defects such as LCD.[8]

Figure 2: Morphokinetic activity of embryos based on 35-day pregnancy outcomes. Only embryos which share a sire are included in this figure to control for variation due to genetic factors. Zero percent of embryos with mean morphokinetic activity > 0.6 established a pregnancy at 35 days post-transfer, showing embryos with high levels of morphokinetic activity produce fewer pregnancies ($P < 0.05$). Significance denoted with the asterisk (*).

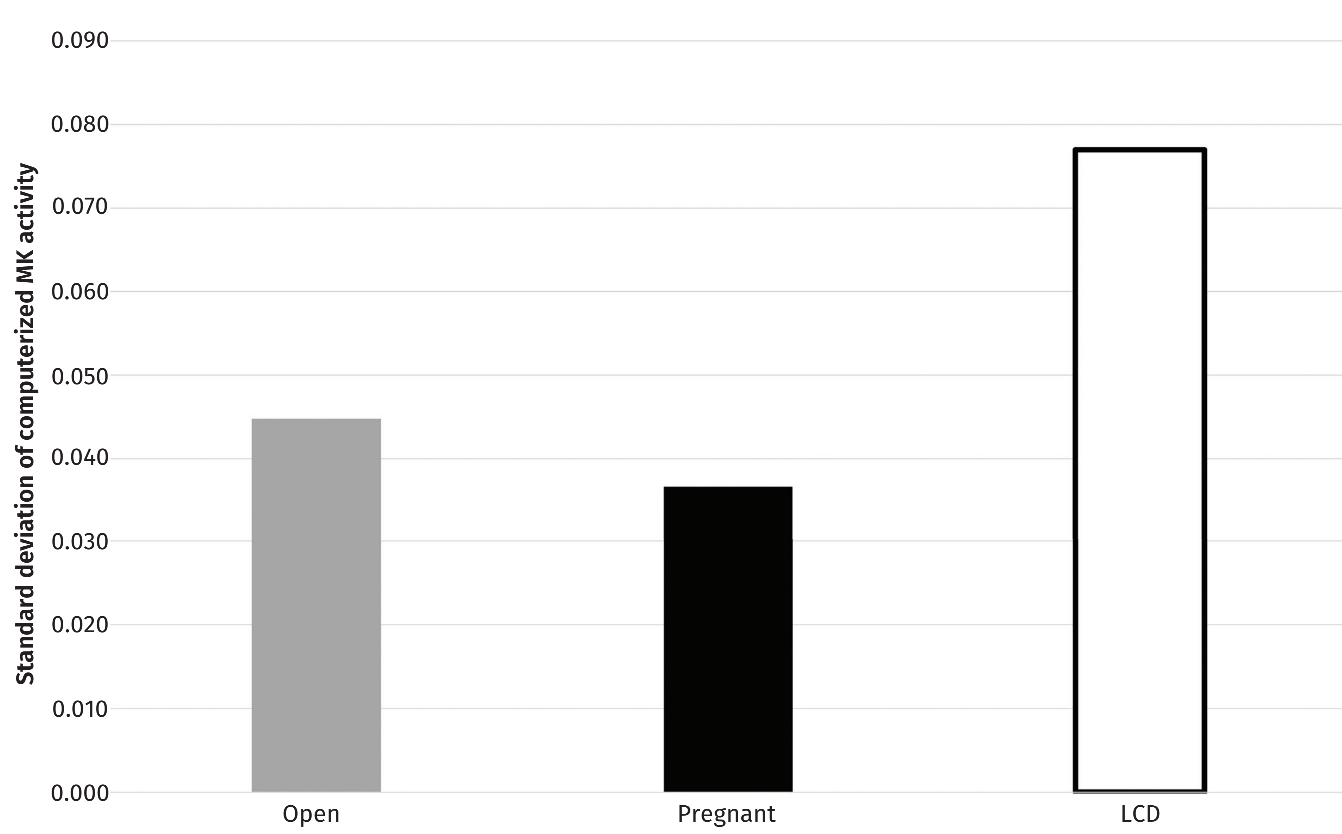


To further investigate the developmental patterns and variations within the embryos, standard deviation of the 90 morphokinetic activity quantifications per embryo was calculated for each individual embryo. These quantifications were recorded 3 times per second over a 30-second video, resulting in 90 total calculations per embryos. The standard deviation was calculated from these 90 measurements of activity, reflecting variation in morphokinetic behavior for each embryo over time, not for the group as a whole. The standard deviations were then compared among embryos that established pregnancies at 35-days post-transfer, embryos that did not establish pregnancies, and the LCD-affected embryo (Figure 3). A Student's t-test was performed to compare embryos that established pregnancies with those that did not, with a significance level set at $P < 0.05$. The LCD affected embryo was excluded from statistical analysis due to the presence of only one embryo in this category. The analysis revealed that the LCD-affected embryo exhibited the highest standard deviation among all embryos that established pregnancies at 35-days post-transfer (Figure 3). Specifically, the standard deviation of the morphokinetic activity for the LCD affected embryo was 0.077, which is double the mean standard deviation of 0.037 observed in the 23 other embryos that established pregnancies (Figure 3).

Discussion

While the case study sample size is limited in scope, the occurrence of LCD in this arrhythmically developing embryo, as determined by the morphokinetic analysis, emphasizes the potential ability of computerized embryo evaluation to detect presence of genetic abnormality in the early pre-implantation embryo, even when initial morphological assessments suggest normal development. Minimum equipment needed to perform this analysis includes a stereoscope with magnification capabilities of 90x and video recording device with minimum resolution of 720p, which can include smartphone cameras. As embryos are microscopic organisms, it is expected that most livestock embryologists and embryo transfer practitioners own comparable equipment. For these purposes, video evaluation of embryos can be implemented in routine livestock embryo transfer practices with minimum hardware investment. Additionally, future mechanisms to automate this analysis on either a web-based platform or smartphone can further increase the accessibility of such analysis, making routine real-time embryo health analysis in the clinic or on farm a reality.

Figure 3: Standard deviation of morphokinetic activity of lethal chondrodysplasia (LCD)-affected embryos was the highest of all 49 embryos, despite sharing a sire. Standard deviation of the lethal chondrodysplasia affected embryo was double that of the other 23 embryos which made pregnancies and delivered genetically normal calves. No statistical differences were found between standard deviation of morphokinetic activity of embryos that made pregnancies and those that did not ($P > 0.05$). The lethal chondrodysplasia affected embryo was excluded from statistical analysis.



Importantly, LCD and other genetic abnormalities are not visibly detectable during the routine embryo morphological analysis, performed on nearly all bovine embryos today. In the case of the LCD-affected embryo in this study, there was a protrusion noted in the embryo's zona pellucida and trophectoderm (Figure 1). Though this abnormality was initially regarded as an inconsequential blemish, the shape of the embryo might have indicated underlying genetic abnormalities affecting skeletal development, although little is known about early embryo formation of LCD-positive calves. The use of video analytics technologies discovered an abnormal embryo development pattern and uncharacteristically high standard deviation, which may be linked to LCD.

While these findings are inconclusive due to the small sample size, previous research suggests that embryo morphokinetic activity may provide a non-invasive assessment of embryo metabolic activity, as embryo morphokinetic activity patterns appear to mimic several trends observed in embryo metabolism studies supporting the Quiet Embryo Hypothesis.[10] Metabolic activity may also reveal critical insights to an embryo's genetic competency. For example, Leese et al. demonstrated that embryos faced with DNA fragmentation and damage allocate significant energy resources (increased metabolic activity) to repair the damage. Therefore, in theory, real-time video analysis of embryos could indicate metabolic function to provide insights to an embryo's health, developmental potential and genetic competency. It is demonstrated that video analysis of embryos is sensitive enough to detect micro changes in the embryos' morphokinetic activity in other studies from these researchers. For example, previous studies demonstrated an increase in embryo morphokinetic activity in response to decreased ambient temperature, suggesting embryos increased their metabolic activity when cold, possibly as a response to the stress.[11]

In this present case study, the LCD-affected embryo demonstrated a low, quiescent morphokinetic activity state with an altered, variable, arrhythmic pattern, including significant increases and decreases from the mean morphokinetic activity level. It was found that the standard deviation of the embryos morphokinetic activity was elevated, compared to other embryos which established pregnancies. When compared to the standard deviation of the other 89 embryos transferred along with the LCD-affected embryo, 7 embryos showed equal or higher standard deviation, all which did not establish a pregnancy at 35-days post transfer. While cause of failed implantation and genetic information about these 7 embryos is unknown, these findings are consistent with data in other studies that suggest high standard deviation of embryo morphokinetic activity is correlated to embryos which will not establish pregnancies that survive to term or result in live birth.[3,4] While inconclusive, it is possible this assessment revealed early insights to the embryo's abnormal genetic composition and exposed dysfunctional developmental patterns which were pertinent to LCD and not compatible with life.

The use of embryo morphokinetic analysis and careful monitoring post-transfer provides valuable insights into embryo viability and potential genetic disorders. Despite the embryo's apparent normal development through early pregnancy, the manifestation of LCD later underscores the unpredictable nature of genetic defects and the challenges of early detection.

Conclusion

Overall, this case report highlights the correlation of LCD in a Holstein embryo following in vitro embryo production and transfer, despite initial normal development and routine monitoring. In this case, the use of embryo video analytics may have found specific features present in genetically compromised embryos, such as embryos bearing LCD, and emphasizes the value that these advanced technologies can bring to studying genetic defects in pre-implantation bovine embryos in the future.

Conflicts of Interest

The authors of this manuscript, Cara Wells, Cameron Hayden, Michael Rea and Russell Killingsworth all declare a financial conflict of interest. Specifically, authors hold stock in EmGenisys, Inc., the company that owns the intellectual property related to the technology described in this article.

Disclosures

In limited cases, ChatGPT-4o was used to help simplify complex sentences and assist with grammar. No AI-assisted technologies were used to generate content.

Authors Contributions

Michael Rea recorded the video of the lethal chondrodysplasia embryo, transferred the embryos, and followed the pregnancy outcomes. Cara Wells prepared the manuscript. Cameron Hayden, Russell Killingsworth and Cara Wells performed the data analytics. All authors contributed to editing the manuscript and were involved in the final approval of the manuscript.

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