Machine-learning models evaluate embryo morphokinetics to detect subclinical signs of heat stress in embryos produced by multiple ovulation embryo transfer

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Introduction

Increased demand for animal-based protein, driven by the increasing world population, has emphasized the use of bovine embryo transfer (ET). However, this demand is predominantly in locations where environmental conditions, like heat stress, are inhibiting reproductive performance. Heat stressed donors have decreased viable blastocyst production, greater variability in embryonic gene expression and lower ET pregnancy outcomes in current literature. These embryonic changes are nonidentifiable to the human eye, highlighting the need for solutions to identify compromised embryos and improve ET efficiencies. Machine-learning artificial intelligence has been used to evaluate health by assessing embryo morphokinetics, or time-specific morphological changes during embryo development, from videos of embryos in standard media. Thus, the objective was to use machine learning to detect real-time morphokinetic activity of embryos from donor cows undergoing multiple ovulation embryo transfer (MOET) based on seasonal exposure to heat stress.

Materials and methods

A retrospective study was performed on 237 bovine embryos (n = 237) from *Bos taurus* donors that underwent MOET in the Texas panhandle region during routine practice field conditions. All embryos were quality grade 1 or 2 according to International Embryo Transfer Society (IETS) standards. A 30 s video was recorded of all embryos using standard equipment, with up to 7 embryos per video. Each 30 s video was recorded at 30 frames per second leading to a total of 900 frames for analysis (30 fps × 30 s video = 900 total frames).

Object recognition, image subtraction and contrast boosting graphic imaging techniques were applied to videos to conclude meaningful data. Computer vision object recognition identified individual embryos, followed by an image subtraction process that quantified pixel changes from one frame to the next, whereby, subtracted frames provided the absolute difference between pixel values. This delivered an objective measurement of embryo morphokinetics. Donors undergoing MOET during the summer (June-August) were considered at risk of potential heat stress, whereas donors collected during the winter (November-February) were not. Data were analyzed using a t-test with a *P*-value of 0.05 for significance.

Results

Results from analysis are presented as normalized activity (standard deviation/mean) pixel changes from embryos in relationship to what season the donor underwent MOET. The average external temperature for the location of donor housing and embryo collection (Shamrock, TX) was approximately 32.8 °C during summer and approximately 12.2 °C during winter collection periods. There is evidence that embryos produced from donors during the summer had greater normalized activity (2.46 ± 0.54) than those produced during the winter (1.98 ± 0.63) ($P < 0.05$).

Significance

Data suggest that there is evidence for machine-learning models to identify differences in real-time embryo morphokinetics, whereby, embryos collected in the summer had greater change, or real-time morphokinetic activity, when compared with those collected in the winter. Heat induces a stress response on embryos, and from this, it is hypothesized that the machine-learning model is depicting change in embryo metabolism. This increased metabolism of the heat stressed embryos has been linked to the embryo trying to repair denatured proteins and damaged DNA. It is important to note that all embryos from this study were morphologically graded acceptable for transfer; yet, damages due to heat stress, combined with increased metabolic activity, suggest that embryos from donors collected in the summer might have lower pregnancy outcomes when transferred. Future work aims to explore pregnancy outcomes. Additional research to noninvasively identify subclinical signs of heat stress in bovine embryos provides a practical application to improve reproductive performance of donors in at-risk environmental conditions.

