Use of image processing techniques can indicate bovine embryo stress and response to temperature changes in real-time

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Introduction

Temperature is a known factor affecting pre-implantation embryo development and should be controlled to prevent a decrease in developmental competence. However, practical considerations of the conventional embryo transfer procedure require the embryos to endure fluctuations in temperature as the embryos are flushed from the in vivo uterine environment, to the in vitro collection dish and into the embryo laboratory. This transition typically involves the embryo culture environment decreasing from 38 °C (body temperature) to approximately 20 °C (room temperature), possibly inducing damage to the embryo and reducing the likelihood of the embryo to result in live birth. The objective of this study was to use graphic imaging and machine learning techniques to measure real-time bovine embryo morphokinetic activity as the embryo transitions from 38 °C to environmental room temperature.

Materials and methods

Ninety-five embryos were flushed from donor Holstein females during routine practice in field conditions. After embryos were located, identified embryos were placed into a holding media. The temperature of the holding media was recorded, and a 30-second video of the embryos were recorded on a View4k camera at a frame speed of 30 frames per second. Environmental room temperature ranged from 33.3 °C to 22.8 °C. Embryos were immediately transferred into eligible recipients and pregnancy outcomes were confirmed with ultrasound.

Graphic image processing techniques were applied to embryo videos, including object recognition, image subtraction and contrast boosting. These methods involve subtracting the digital numeric value of each pixel from the value of the corresponding pixel in the previous frame, to generate an output which is the absolute difference between pixel values. This technique allows the objective measurement of the morphokinetics of the embryo over time by quantifying pixel change.

This measurement was recorded for each subsequent frame for the entire duration of the video (30 seconds x 30 fps = 900 total frames) for each embryo. Mean pixel change per embryo was calculated and compared to media temperature.

Results

It was found mean pixel change is positively correlated to a decrease in temperature showing embryos have a higher mean pixel change, thus more morphokinetic activity, at lower temperature than higher temperatures ($r = 0.7$). Morphokinetic activity is highly correlated at 30 °C-33 °C and 22.8 °C-23.8 °C ($r > 0.8$). Morphokinetic activity demonstrated a greater range and more variability per embryo at 25.5 °C-28.8 °C ($r < 0.4$).

Significance

This data demonstrates an increase in bovine embryo morphokinetic activity as media temperature decreases, suggesting cooler temperatures induces embryo stress which can be detected as a rise in morphokinetic activity. It is hypothesized that this increase in morphokinetic activity is due to an increase in embryo metabolism as the embryo attempts to maintain homeostasis in the changing environment. Interestingly, there was more variability of morphokinetic activity in embryos in media temperatures 25.5 °C-28.8 °C, with some embryos possessing the ability to maintain a more quiescent state and others demonstrating a sharp spike in increased morphokinetic activity, suggesting this temperature range is an inflection point that triggers a response in some embryos. When pregnancy outcomes were compared, embryos which maintained a more quiescent state during the inflection point established more pregnancies ($P < 0.05$). Data suggest these techniques can be used to detect subclinical signs of embryonic stress and select hearty embryos robust to temperature fluctuations.

