Analysis of short videos of bovine embryos can detect presence of cryodamage

R. Killingsworth,[1] BS, DVM; C. Hayden,[1] BS, MS; S. Hickerson,[2] BS, MS; J. Webb,[1] BS, MS, PhD; A. Wiik,[1] BS, MS; J. Gibbons,[2] BS, MS, PhD

[1]Shamrock Veterinary Clinic, Shamrock, TX 79079
[2]Texas Tech University School of Veterinary Medicine, Amarillo, TX 79106

Introduction

Cryopreservation of bovine embryos has transformed animal breeding strategies by allowing storage of genetic material which enables efficient recipient utilization, sale and marketing of embryos, and shipping and transport of embryos around the world. Advancements have been made in cryopreservation techniques including the introduction of direct transfer ethylene glycol media, controlled rate freezers allowing precise temperature control of the freezing curve and vitrification techniques. However, little advancement has been made to evaluate embryo survival of cryopreservation which results in the transfer of embryos which have suffered cryodamage and have no chance of establishing a pregnancy. The objective of this study was to use graphic image processing (GIP) and machine-learning (ML) techniques to evaluate embryo health post-cryopreservation.

Materials and methods

Thirty-two bovine embryos were frozen either in glycerol (n = 16) or ethylene glycol (n = 16) in previous years during routine processing of embryos. Embryos were thawed according to standard protocol and placed individually into a multi-well plate with holding culture media. Embryos were allowed to rehydrate for 1 hr in standard culture conditions. Embryos were graded based on stage and quality according to IETS standards. Thirty-second videos of each embryo were recorded with a smartphone. Embryos were returned to standard culture conditions and re-evaluated for stage and quality grade at 24 h post-thaw.

Video Analytics:
1) Videos were processed with GIP techniques to quantify frame-by-frame pixel change throughout the 30 s video duration. This allows embryo morphokinetic activity to be measured over time, which was presented as normalized activity (standard deviation/mean) pixel change.

2) Videos were analyzed by EmGenuity Software (EmGenisys, USA), an ML software which casts a viability prediction frame-by-frame to objectively predict an embryos likelihood to result in pregnancy. ML model prediction accuracy was determined by comparing predicted outcomes to actual embryo development outcomes.

Results

Eleven out of 16 (68.75%) embryos frozen in glycerol continued in development. Eight out of 16 (50%) embryos frozen in ethylene glycol continued in development. None of the embryos (0%) hatched from the zona pellucida, which is considered normal development for a viable embryo post-thawing. When videos were processed with GIP techniques to measure embryo morphokinetic activity, embryos which continued development had more activity than embryos which stalled in development ($P < 0.05$). Additionally, embryos frozen in glycerol had higher morphokinetic activity, through results were not significant ($P < 0.05$). When videos were analyzed by EmGenuity Software, the software predicted 100% of embryos to be non-viable.

Significance

Embryos in this study appeared to be compromised, as none of them hatched from the zona pellucida post-thaw. However, the analyzed video results suggest both GIP techniques and ML software can be utilized to predict embryo survival of cryopreservation. When videos were analyzed to measure morphokinetic activity, embryos which continued to develop demonstrated more activity, likely because these embryos were undergoing metabolic processes to progress to more advanced developmental stages. The ML software EmGenuity was 100% accurate in predicting embryo viability, as none of the embryos demonstrated the ability to hatch from the zona pellucida. Future work aims to increase study sample size to develop further outcomes to determine the ability of GIP and ML software to detect embryo cryodamage subclinically and improve pregnancy outcomes of bovine embryo transfer.

