Lipid content and cryopreservation of Jersey cattle embryos
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Cryopreservation of in vivo derived Jersey bovine embryos have resulted in 10% lower pregnancy rate compared to other dairy breeds. Poor embryo survival after cryopreservation has been partially attributed to the high lipid content of Jersey cattle embryos. We hypothesized that the lipid content of in vivo and in vitro produced (IVP) Jersey embryos is higher than respective Holstein embryos. The objectives of this experiment were (1) to analyze lipid content of in vivo and IVP Jersey cattle embryos and (2) evaluate Jersey IVP embryo survival rates after three cryopreservation procedures. For experiment one, IVP embryos (n=60) were produced by standard procedures, briefly oocytes were aspirated from 2 to 8 mm follicles from slaughterhouse ovaries then matured for 24h in SMM medium (BoviPro, MOFA Global, Verona, WI). Matured oocytes were fertilized using semen from two different bulls for each breed, and embryos were cultured in BBH7 medium (BoviPro, MOFA Global, Verona, WI) at 38.5°C in 5% O2, 5% CO2, and 90% N2. In vivo produced embryos (n=27) were collected by standard procedures 7 days after artificial insemination. The lipid content of embryos was quantified by staining Day 7 blastocysts with 1 μg/mL Nile red dye (580-596nm), after which a digital photograph of the equatorial region of the embryo was taken at 40x, and fluorescence intensity (FI) was measured with Image Pro software. Comparisons within and between breeds were evaluated by T-Test. For experiment two, Grade 1 Jersey IVP blastocysts (n=356) were divided into six treatments using a 2x3 factorial design comparing intact (IB) vs collapsed blastocoel (CB) and three cryopreservation methods: slow freezing (SF) vs vitrification using open pulled straws (OPS) or cryotop (CT). Slow freezing embryos were equilibrated in 0.7 M glycerol and 0.1 M galactose in holding media for 5 min, held for 10 min at -6°C, seeded after 5 min, decreased to -32 °C at 0.5 °C /min and, held at -32°C for 5 min, and plunged into liquid nitrogen. Vitrified embryos were equilibrated in 1.5 M ethylene glycol (EG) for 5 min, exposed to 7 M EG + 0.6 M galactose for 30 s while loaded into OPS or placed onto CT, then immediately plunged into liquid nitrogen. SF embryos were thawed in air for 10 s and placed in a water bath at 37°C for 45 s. Vitrified embryos were warmed directly into holding medium at 37°C supplemented with 1.0 M, 0.5 M and 0.25 M galactose for 3 minutes each. Subsequently, embryos were cultured in BBH7 and re-expansion rates were assessed at 24 and 48h after warming and data were evaluated by GLIMX. For experiment 1, Jersey and Holstein IVP embryos had higher lipid content than Holstein in vivo produced embryos (56, 55 vs 43 ± 4 FI; p<0.05), but were not different than Jersey in vivo-derived embryos (49 ± 4 FI; p>0.1). For experiment 2, re-expansion rates were higher for CT, than OPS, and SF (85 vs. 66 vs. 72% ± 0.4, respectively; p<0.05). Main effect means for re-expansion were higher for CB than IB (79 vs 68% ± 0.3; p<0.05). In conclusion, IVP embryos have higher lipid accumulation over Holstein in vivo embryos. The CT method and collapsing the blastocoele prior to cryopreservation resulted in higher blastocyst survival rate. Further studies including transfer of embryos to recipients are necessary to corroborate these results.

Keywords: Lipid, cryopreservation, Jersey, embryo, Nile red