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86 IMPROVED BOVINE EMBRYO PRODUCTION USING NOVEL *IN VITRO* CULTURE SYSTEMS

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Abstract

Development and testing of new embryo production components is important to improve the outcome following *in vitro* production of bovine embryos. The objective of this study was to compare media used in two bovine embryo production systems (control and EmbryoTrans Biotech: ETB). In Exp. 1, abattoir-derived cumulus-oocyte complexes were randomly assigned and *in vitro* matured (IVM) in either control [Medium 199 with Earles salts (Invitrogen, Carlsbad, CA, USA), supplemented with 10% fetal bovine serum (Hyclone, Logan, UT, USA), 1% penicillin/streptomycin (Invitrogen), 0.2 mM sodium pyruvate, 2 mM L-glutamine (Sigma Chemical Co., St. Louis, MO, USA), and 5.0 μg mL^{-1} of Folltropin®-V (Vetoquinol, Pullman, WA, USA)] or ETB BO-IVM medium for 21 to 24 h. IVF was conducted in 500 μL of pre-equilibrated modified Tyrode-lactate medium for control (Pryor *et al.* 2011 Theriogenology **75**, 24–33) or ETB BO-IVF in Nunclon® 4-well multi-dishes (VWR Scientific, Pittsburgh, PA, USA). Seventeen hours post-insemination, presumptive zygotes were cleaned of cumulus cells and cultured in either Bovine Evolve (Zenith Biotech, Guilford, CT, USA) supplemented with 4 mg mL^{-1} of Probumin BSA (EMD Millipore, Norcross, GA, USA), under oil (Irvine Scientific, Santa Ana, CA, USA) or ETB BO-IVC medium under BO-oil for 7 days (8 days post-IVF). All cultures were performed at 38.5°C in a humidified atmosphere of 5% CO_2, 5% O_2, and 90% N_2 using BT37 incubators (Planer Plc, Sunbury, UK). For Exp. 2, all conditions were maintained except a modified ETB BO-IVCA medium was used. On Day 8 of IVC, grade 1 and 2 blastocysts (BL) through hatching blastocysts (HBL) were counted and used to calculate total viable rates. In Exp. 2, these embryos were fixed in cold methanol, washed in PBS/0.1% Tween 20, mounted in 10 μg mL^{-1} Hoechst 33342/glycerol, and viewed under UV light to count cells ($n = 49$ and 107 for control and ETB, respectively). Each experiment was replicated 3 times with a total of 425 oocytes in Exp. 1 and 430 in Exp. 2, divided equally between treatments. Percentage data were transformed using arcsine square root function before analysis and means compared using a paired Student's *t*-test. For Exp. 1, there were no differences in rates of cleavage or viable embryos between control and ETB systems (81.3% and 42.9% *v.* 80.5% and 48.4%, respectively). In Exp. 2, ETB was superior to control for percent viable, HBL, and combined HBL/expanded BL (51.9, 23.9, 45.8% *v.* 29.2, 5.8, 20.5, respectively; $P < 0.05$). Differences between mean cell counts for viable embryos were significant (control = 127.0 ± 6.7 s.e.m. and ETB = 162.7 ± 5.7; $P < 0.0001$). Embryo viability decreased in control media between Exp. 1 and 2 (42.9 *v.* 29.2%; $P < 0.05$). Seasonal differences may have contributed via heat stress with temperatures ranging from 23.8°C for Exp. 1 to 33.8°C for Exp. 2. Interestingly, embryo development in the ETB media did not decrease under the same conditions. In conclusion, ETB media produced more high-quality embryos than control under varying conditions experienced by commercial IVF companies.