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141 BOVINE EMBRYO DEVELOPMENT RATES ARE AFFECTED WHEN OOCYTES ARE MATURED IN DIFFERENT VIALS CONTAINING HEPES/BICARBONATE BUFFERED MEDIUM

N. Hashem [A], J. O. Secher [B], J. H. Pryor [C], C. R. Long [D], C. R. Looney [C], B. Avery [A], P. Hyttel [B] and L. Stroebech [A]

+ Author Affiliations

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Abstract

Laboratory ware for the *in vitro*-produced embryos is generally made from embryo-tested plastic instead of glass. The quality of the plastic is crucial for the outcome because plastic is often toxic to gametes (Nijs *et al.* 2009 Fertil. Steril. **92**, 527–535). In addition, gas molecules permeate through the plastic at a rate that depends on a variety of factors, such as diffusion coefficient and thickness of the plastic. In an incubator with appropriate concentration of CO_2 and vented culture vessels, the gas permeability of the plastic is not important. When oocytes are transported outside a controlled atmosphere, gas permeability, toxicity, and oocyte cumulus cell CO_2 metabolism could perturb the outcome. Medium containing bicarbonate buffer increases pH outside of a controlled atmosphere within minutes, whereas medium buffered with HEPES maintains suitable pH for hours. Previously, we tested that gas permeability differs among plastic vials and glass vials with no cumulus-oocyte complexes (COC) by measuring pH after 2, 5, and 24 h at the same temperature. The objective of this study was to compare pH post-maturation, blastocyst development rates on Day 8 post-IVF (Day 0 = IVF) between 2 different 1.2-mL polypropylene cryovials (A: VWR DK, 479-1219; B: Sigma-Aldrich, St. Louis, MO, USA, CLS430289), glass vial (VWR DK, NSCAC4015-96), and 4-well plate (4WP) as control (Thermo Fisher Scientific, Waltham, MA, USA, 144444). A total of 1135 abattoir-derived COC in Exp. 1 and 133 in Exp. 2 were divided equally between the treatments (20–25 COC per vessel). Vials/4WP contained 0.8/0.5 mL of BO-IVM HEPES, a HEPES/bicarbonate medium (IVF Bioscience; BO-HEPES-IVM, UK). Maturation lasted 22 to 24 h at 38.8°C in an incubator with either a humidified atmosphere of 5.5% CO_2 in air (Exp. 1) or with no CO_2 contact (Exp. 2). In Exp. 1, oocyte vials were matured without a vial lid while in Exp. 2 vial lids were closed. Statistical analysis was performed with chi-square and mean ± SD. In Exp. 1, Day 8 blastocyst rates were evaluated as percentage of inseminated oocytes, with 4WP and glass vials significantly higher than cryovials A or B (38 ± 8.9%, 35 ± 7.5% *v.* 26 ± 3.2%, 26 ± 3.5%; respectively; $P < 0.05$). In Exp. 2, pH was measured for the 3 vials immediately post-maturation. Day 8 blastocyst rates were significantly higher in the glass vials as compared with cryovials A and B (pH 7.26, 31%; pH 7.60, 20% and pH 7.72, 22%, respectively; $P < 0.05$). In conclusion, blastocyst rates are affected by type of vial, as well as different gas permeability among other factors influencing pH. Further studies are necessary to optimize the maturation of the oocytes in HEPES-buffered media.