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Development of a simple, portable carbon dioxide incubator for in vitro production of bovine embryos

T. Suzuki [a,*], C. Sumantri [a,b], N.H.A. Khan [c], M. Murakami [a], S. Saha [a]

[a] *United Graduate School of Veterinary Sciences, Yamaguchi University, Yamaguchi 753, Japan*
[b] *Faculty of Animal Science, Bogor Agricultural University, Bogor, Indonesia*
[c] *Department of Animal Husbandry and Veterinary Services, Government of Karnataka, Bangalore 560001, India*

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Abstract

The objective of this study was to develop a simple and portable CO_2 incubator using effervescent granules (EG) and to examine the effect of negative and positive air pressure for in vitro maturation (IVM), fertilization (IVF) and culture (IVC) of bovine oocytes. In experiment 1, cumulus-oocyte complexes (COCs) were matured (22 h), fertilized (5 h) and cultured (7 days) using 0.25, 0.5 or 1.0 g of EG per 0.6 l added to maintain an optimum level of CO_2 (approximately 3, 6 or 12%, respectively) for in vitro production of embryos. Control oocytes, zygotes and embryos were cultured in a standard CO_2 incubator. The blastocyst production rates observed on Days 7 to 9 after insemination were $20.5 \pm 4.2\%$, $18.5 \pm 3.9\%$ and $28.7 \pm 5.1\%$ for the 0.25 g EG, 0.5 g EG treatments and control, respectively. These rates were significantly higher ($P < 0.05$) than that of the 1.0 g EG treatment ($8.7 \pm 2.6\%$). The number of cells in the inner cell mass (ICM) and trophectoderm (TE) produced from blastocysts using the control procedure were 40.8 ± 2.9 and 81.2 ± 5.3, respectively, and were higher ($P < 0.05$) compared to the 0.50 g EG (34.6 ± 2.9 and 66.8 ± 5.7) and 1.0 g EG treatments (33.4 ± 3.4 and 67.2 ± 7.3). In experiment 2, COCs were placed in a small box with 0.25 g of EG so that the effects on IVM, IVF and IVC of positive or negative air pressure could be compared. The blastocyst production rate observed in the negative air pressure treatment ($29.6 \pm 4.6\%$) was higher ($P < 0.01$) than that of the positive air pressure treatment ($6.2 \pm 1.5\%$) or the normal treatment pressure ($P < 0.05$; $18.7 \pm 4.2\%$) but did not differ from that of the control ($30.7 \pm 4.4\%$). These results indicate that this simple type of

* Corresponding author. Tel./fax: +81-839-33-5935; e-mail: suzuki@agr.yamaguchi-u-ac.jp

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