DEVELOPMENT OF A SIMPLE, PORTABLE CARBON DIOXIDE INCUBATOR FOR PRODUCTION OF BOVINE IVF EMBRYOS

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We have developed a simple, economical and portable CO2 incubator for production of bovine IVF embryos in the field. The incubator consists of a metallic chamber (29L x 26W x 21H cm); the bottom plate of this chamber is electrically heated to maintain the chamber temperature between 38.5 and 40.0°C. Incubations are carried out in a small plastic box (15L x 10W x 4H cm) placed inside this chamber. The cover of this box is transparent to view the culture dishes and has an inlet at one end. Immediately below this inlet, at the bottom of the box, is a plastic dish (3 cm diameter) for placing effervescent granules. Distilled water (5 ml) is added through the inlet into the plastic dish containing effervescent granules to generate CO2, and the inlet is capped with a silicone stopper to maintain constant CO2 level inside the box. To examine the amount of effervescent granules that should be added to maintain an optimum CO2 levels for successful in vitro production of embryos, 0.25, 0.5 and 1.0g of effervescent granules (Tartaric acid, 420 mg; Carbonated hydrogen natrium 460 mg; Silicone fiber 10 mg per g of effervescent granules) were tested. Blastocyst production rate and the number of ICM and trophectoderm cells of the blastocysts produced were compared among treatment groups, as well as with embryos developed in a Sanyo CO2 incubator (Control:Sanyo electric Co. LTD., Osaka, Japan). Procedures for in vitro maturation of oocytes, in vitro fertilization and in vitro culture of cleaved embryos were as reported previously (Suzuki et al., Theriogenology 34:1051, 1990); and all step of IVM, IVF and IVC were done in both incubators. More than 250 immature oocytes were allocated at random to each treatment. Immunosurgery and a differential staining techniques (Iwasaki et al., J. Reprod. Fertil. 90:279, 1990) were used to determine ICM and trophectoderm cells. Results are shown in Table 1. The number of ICM and trophectoderm cells from blastocysts produced in the control group were 30.1±6.6 and 79.3±21.3, respectively and were not different (P>0.05) from the other groups. These results suggest that the simple incubator developed in our laboratory can be successfully used for production of bovine IVF embryos and could be used at the farm level.

Table 1. In vitro embryo development on day 7 after insemination in newly developed and standard (control) incubators

Parameter	New incubator			Control
	0.25g*	0.50g*	1.00g*	
No. of oocytes	257	335	249	281
No. developed to cleavage	151[a] (58.8%)	188[a] (56.1%)	90[b] (36.1%)	151[a] (53.7%)
No. developed to blastocyst	42[a] (16.3%)	39[a] (11.6%)	12[b] (4.8%)	43[a] (15.3%)
Total embryos	193[a] (75.0%)	227[a] (67.8%)	102[b] (41.0%)	194[a] (69.0%)

[a,b] Values within rows with different superscripts differ (P<0.05).

* Amount of effervescent granules added to generate CO_2 inside the newly developed incubator.