

Development of Ovine Embryos Derived from Oocytes Matured *in vitro* in the Absence of CO₂

Margawati, E.T.¹⁾ I. Supriatna²⁾ T.L. Yusuf²⁾ and Y. Supriondho³⁾

1. Research and Development Centre for Biotechnology-LIPI, Indonesia
2. Animal Reproduction and obstetrics Department, Faculty of Veterinary Medicine, IPB. Bogor, Indonesia
3. Animal Production Department. Faculty of Animal Science, Diponegoro University, Semarang, Indonesia

The purpose of the study was to determine the effect of CO₂ during *In Vitro* Maturation (IVM) and subsequent development into morula/blastocyst stages in sheep and to develop a method of in vitro maturation in ruminant animals.

Oocytes from abattoir ovaries were collected by methods of aspiration and spraying of media using a 18-G needle with aspiration medium of hepes-199 (H199) + 0.4% BSA + 50 i^g/ml Heparin. The oocytes were divided into 3 groups and treated separately as follows: T1) oocytes were cultured in an eppendorf containing maturation medium (Bicarbonate-199 + 10% FCS + 10 i^g/ml FSH + 10 j^g/ml hCG + 1 i^g/ml Estradiol) overlaid with mineral oil. The IVM medium was equilibrated in 5% CO₂ incubator prior to culture of the oocytes then matured in the absence of CO₂; T2) oocytes were; T3) oocytes were cultured in a petri dish containing maturation drops (as T1 medium) overlaid with mineral oil it was equilibrated in 5% CO₂ incubator prior to culture then matures in 5% CO₂ incubator. All cultures were maintained at 38°C in a humidified incubator for 24 hours. The mature oocytes were fertilized in BO (Brackett and Oliphant) medium with concentration of approximately 12 x 10⁶ sperm/ml for 6 hours and cultured in synthetic oviductal fluid (SOF) supplemented with amino acids (AA) and bovine serum albumin (BSA) at 38°C in humidified incubator with 5%CO₂ for 7 days.

The result showed that there was a highly significant effect of CO₂ during oocytes maturation on the percentage of cleaved oocytes (P<0.01). However, oocytes matured in the absence of CO₂ did not affect on either the percentages of morula/blastocyst or the rates of morula/blastocyst. This study suggests that ovine embryos derived from oocytes matured *in vitro* in the absence of CO₂ can be developed *in vitro* morula or blastocyst stages.