In Vitro Maturation and Fertilization of Ovine Oocytes in a System with Absence of 5% CO_2

Jaswandi, Arief Boediono and Muhammad Agus Setiadi

Faculty of Veterinary Medicine, Bogor Agricultural University,Darmaga Bogor, Indonesia

ABSTRACT. **In vitro maturation and fertilization of ovine oocytes in system with absence of CO_2 was studied. The cumulus oocyte complexes (COCs) were matured in four different maturation medium, namely: a). TCM-199 only, b). TCM-199 +10 mM Hepes, c). TCM-199 + 20 mM Hepes and d). TCM-199 + 30mM Hepes. The COCs were incubated in the incubator with 5% CO_2, incubator without 5% CO_2 and incubator with effervescent granule (EG) as the source of 5% CO_2 at 38.5°C for 24 hours. Four different time of incubation (20, 24, 28 and 32 hours) in system without 5% CO_2 was design to get the maturation rate. Treatments above were used to mature the oocytes for in vitro fertilization. The treatment of fertilization consists of the COCs fertilized for 18 hours in three incubator systems. The maturation rates of COCs in the incubator without 5% CO_2 or EG gradually increased by increasing of Hepes level from 0 mM to 20mM, whereas the maturation rates in the incubator with 5% CO_2 was decreased by increasing Hepes level in medium. However, they were not significantly different among the treatments, but there was the interaction between using of Hepes and incubator system. The level of 0 mM Hepes in the incubator without 5% CO_2 and 30 mM Hepes in the incubator with 5% CO_2 had the lowest maturation rate. The optimal maturation rate was obtained when COCs were incubated for more than 28 hours. The fertilization rate was not affected by incubator system. These results concluded that in vitro maturation and fertilization of ovine oocytes could be done in the incubator without 5% CO_2 by adding the 10-20 mM Hepes buffered into TCM-199 medium.**

Key words: IVF, ovine, 5% CO_2, Hepes, effervescent granule.

Introduction

The successful of embryo production is influenced by many factors such as the oocyte quality, medium and culture system. The optimum environment conditions are temperature 35–39°C and 5% CO_2 in air (Gordon, 1994). The CO_2 is required to maintain pH.

To increase the flexibility of embryos production in vitro some researcher tried to substitute the role or the source of CO_2. De Smed et al (1992), Le Gal (1995), Martino et al (1995) has successfully matured the goat oocytes in absence of 5% CO_2 in the incubator by using Hepes buffered medium. The production of cattle embryo in the incubator without 5% CO_2 has also reported by using effervescent granule (EG) as CO_2 sources (Suzuki et al, 1995; Khan et al, 1997; Suzuki et al, 1999)

The purpose of this research was to develop a method of in vitro maturation and fertilization of ovine oocytes in the absence of 5% CO_2 system, so that it will be possible to mature oocyte out side of laboratory. Moreover, it will increase the efficiency of utilization oocytes resources in the slaughterhouse or to be a model in application *dangerous animal* oocytes to yield the in vitro embryos.

Materials and Methods

Oocytes collection. Ovaries were collected from the slaughterhouse and brought to laboratory in physiological saline (NaCl 0.9%) supplemented with 100 mg/ml streptomycin and 100 IU/ml penicillin at 35°C within 5 hours. Ovaries were washed 3 times, and their oocytes were collected by slicing method in PBS solution (Nissui, Japan). Only oocytes with a homogenous cytoplasm and surrounded with one or more cumulus complete were selected for maturation.

In vitro maturation. After twice washing, the oocytes were matured in four different maturation media namely TCM-199 only (plus 0 mM Hepes), TCM-199 + 10 mM Hepes, TCM-199 + 20 mM Hepes and TCM-199 + 30mM Hepes. All media supplemented with 10% Calf Serum (CS, Sigma, St Louis USA), 10 mg/ml Follicle Stimulating Hormone (FSH, Sigma, St Louis USA) and 50 mg/ml