PROCEEDINGS OF

THE INDONESIAN BIOTECHNOLOGY CONFERENCE

Volume II

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Challenges of **BIOTECHNOLOGY** in the 21st Century

Organized by

The Indonesian Biotechnology Consortium

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Preface from Editors

More than one year after the IBC'97 was held, and after working hard, the Proceedings Team of the IBC'97 Organizing Committee is finally finishing her task and a proceedings of The Indonesian Biotechnology Conference 1997 (IBC'97) is now ready to be published and circulated among biotechnologiest around the world.

The proceedings consists of two volumes. The volume 1 covers papers on Industrial Biotechnology while the volume 2 contains papers on Agricultural, Forestry, and Medical Biotechnologies.

On behalf of the Proceedings Team, I would like to express my sincerely thank to all members of the Steering as well as the Organizing Committee of the IBC'97 for their encouragement, suggestions, and help so that this effort could materialized.

I do believe that this proceedings of the IBC'97 would enrich our knowledge in biotechnological field, and in turn it could materialized.

I do believe that this proceedings of the IBC'97 would enrich our knowledge in biotechnological field, and in turn it could benefit to mankind.

Thank you very much,

DR. Umar A. Jenie
Chief Editor

Development of ovine embryos derived from oocytes matured *in vitro* in the absence of CO_2*

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ABSTRACT

The purpose of the study was to determine the effect of CO_2 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 (H 199) + 0.4% BSA + 50 µg/ml Heparin. The oocytes were divided into 3 groups and treated separately as follows: T1) oocytes were matured in an eppendorf containing maturation medium (B 199 + 10% FCS + 10 µg/ml FSH + 10 µg/ml hCG + 1 ug/ml Estradiol) overlaid with mineral oil. The IVM medium was equilibrated in 5%CO_2 incubator for 2 hours prior to maturation then incubated in the absence of CO_2; T2) oocytes were matured in maturation drops in a petri dish overlaid with mineral oil and incubated in the absence of CO_2; T3) oocytes were matured in maturation drops in a petri dish (as T2) overlaid with mineral oil. The maturation drops were equilibrated in 5%CO_2 incubator for 2 hours prior to maturation then incubated in 5%CO_2 incubator. All maturations were maintained at 38°C in a humidified incubator for 24 hours. Fresh sperm were capacitated in Hepes TALP. The IVF medium was Fert TALP + penicillamine + Hypotaurine + Heparin. The oocytes were cultured in synthetic oviductal fluid (SOF) supplemented with amino acids (AA) and bovine serum albumin (BSA) at 38°C in a humidified incubator with 5% CO_2 for 6-7 days.

The result showed that there was a highly significant effect of oocyte matured in the absence of CO_2 on the percentage of cleaved oocytes ($P<0.01$). However, oocytes matured in the absence of CO_2 did not affect on either the percentage of morula/blastocyst or the rate of morula/blastocyst. This study suggests that ovine embryos derived from oocytes matured in vitro *in the absence of CO_2 can be developed* in vitro *through morula or blastocyst stages.*

INTRODUCTION

Availability of ovaries is the basic material in commencing of in vitro fertilization (IVF) work. To obtain ovaries from abattoirs in a large number, it is not easy task for several places in Indonesia. When the ovaries can be collected in a large number, consequence, the abattoir

fertilization. This study was also directed to develop a method of in vitro maturation of ruminant oocytes.

MATERIALS AND METHODS

Collection of Oocytes.

Ovine ovaries were obtained from unidentified ewes at local abattoirs and transported to the laboratory in 0.9% NaCl at 30°C in a flask thermos. Oocytes were aspirated and sprayed with a 18-gauge needle attached to a 10-ml disposable syringe. Aspiration medium was Hepes buffered 199 (H199) + 0.4% Fetal Calf Serum (FCS) + 50 µg/ml Heparin. Oocytes were selected under a dissecting microscope and transferred into a petri dish containing oocyte handling medium (H199 + 10% FCS), washed in Bicarbonate buffered 199 medium (B199) supplemented with 10% FCS. Prior to maturation, the oocytes were rewashed in maturation medium (IVM medium).

In Vitro Maturation.

Maturation medium was B199 supplemented with 10% FCS + 10 µg/ml FSH + 10 µg/ml hCG + 1 µg/ml Estradiol (E2). Methods of oocyte maturation were used as treatments. Treatment 1 (T1), oocytes were matured in an eppendorf containing IVM medium and overlaid with mineral oil, prior to culture of the oocytes, the eppendorf was equilibrated in 5% CO_2 incubator at 38°C for 2 hours then incubated in the absence of CO_2. Treatment 2 (T2), oocytes were matured in IVM drops of a petri dish overlaid with mineral oil then incubated in the absence of CO_2. Treatment 3 (T3), oocytes were matured in IVM medium drops of a petri dish overlaid with mineral oil, the drops were equilibrated prior to culture of the oocytes (as T1) then incubated in 5% CO_2. All treatments were held at 38°C for 24 hours in air with a high humidity.

In Vitro Fertilization.

Fresh semen from a local sheep was used in the experiments. The fresh semen was diluted with Hepes TALP'medium. Prior to insemination, a highly motile fraction of sperm was collected by twice centrifugations in Hepes TALP, first at 1800 rpm for 7 minutes, secondly

(T2) or introduced in CO_2 for 2 hours (T1). Overall, the proportion of oocytes reaching MII was less than 50%. This result was low compared to that of IVM of ovine oocytes which matured in a portable incubator of the absence of CO_2 (60%; Byrd *et al.*, 1995). It seems that the role of CO_2 during in vitro maturation of oocytes is likely not to be a crucial requirement during IVM in term of achievement of the metaphase II from immature ovine oocytes. A recent study reported that there was no different among concentrations of 2.5; 5 and 10% CO_2 during IVM on bovine oocytes achieving MII (Pinyopummintr and Bavister, 1995). With regard to O_2, they reported that a low concentration of O_2 (5%) during IVM/IVF drastically decreased the proportions of oocytes reaching metaphase II and total fertilization. In addition, the percentage of polyspermy was markedly increased when IVF was conducted in low concentration of O_2.

Table 1. Effect of gas atmosphere CO_2 during in vitro maturation of ovine oocytes in the achievement of MII (Mean ± SEM)

Treatment	Number of Oocytes	Metaphase II (%)
2 h of CO_2 (T1)	33	39.40±3.34[a]
absence of CO_2 (T2)	38	28.95±2.78[a]
presence of CO_2 (T3)	33	48.49±4.03[a]

No significant difference between treatments (P>0.05)

Development of ovine oocytes derived from maturation in the absence of CO_2 following in vitro fertilization (IVF) using fresh semen was determined in term of cleavage, morula or blastocyst. Percentage of cleavage, morula/blastocyst and rate of morula/blastocyst were analyzed to illustrate the development of ovine oocytes derived from oocytes matured in the absence of CO_2 during IVM (Table 2). Cleavage was recorded at the day-2 following IVF while morula or blastocyst was recorded at day 6 or 7 of IVF. The previous study stated that the absence of gas CO_2 during in vitro maturation did not affect to the achievement of immature oocytes to the number of metaphase II. However, the percentage of cleavage oocytes showed significantly difference among treatments (P<0.01) while there was no significant difference on the percentage of morula/blastocyst and rate of morula/blastocyst (P>0.05). Data of Table

ovaries are obtained from a long distance. It suggests that oocytes can be matured in the absence of CO_2 on the way to the IVF laboratory without influencing subsequent development of embryos.

REFERENCES

Byrd, S.R., G. Flores-Foxworth and M.E. Westhusin. 1995. Normal ovine embryo development following *in vitro* oocyte maturation in a portable incubator in the absence of CO_2. *Theriogenology* **43**: 179 (Abstract).

Margawati, E.T., I. Supriatna, T.L. Yusuf, Y. Supriondho and T. Hastuty . 1997. *In vitro* maturation of ovine oocytes in the absence of CO_2. *Proceedings of Agricultural Biotechnology* (In Press).

Pinyopummintr, T. and B.D. Bavister. 1995. Optimum gas atmosphere for in vitro maturation and in vitro fertilization of bovine oocytes. *Theriogenology* **44**: 471-477

Tervit, H.R., D.G. Whittingham and L.E.A. Rowson. 1972. Successful culture *in vitro* of sheep and cattle ova. *J. Reprod. Fert.* **30**: 493-497.

Thompson, J.G.E., A.C. Simpson, P.A. Pugh, P.E. Donnelly and H.R. Tervit. 1990. Effect of oxygen concentration on in vitro development of preimplantation sheep and cattle embryos. *J. Reprod. Fert.* **89**: 573-578.

Whitten, W.K. 1971. Nutrient requirements for the culture of preimplantation embryos *in vitro*. *Adv. Biosc.* **6**: 129-141.