THE QUALITY OF OOCYTE RESULTED FROM IN VITRO GROWTH (IVG) CULTURE SYSTEM OF INDONESIAN LOCAL CATTLE

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Abstract

The in vitro growth (IVG) culture system of domestic species have been developed. It is expected to provide a new source of a good quality of oocytes for in vitro maturation (M-II) oocyte. The aims of this research are to study the potential oocyte resource of local Cattle of Madura and Bali using in vitro growth method of preantral follicle both for research purposes as well as for in vitro culture system for embryo production. Preantral folicle are main source of oocyte as an inportant tool fo preserve female gamet .Methods use in this research is experimental. Folicles were isolated, cutured in vitro for 14 days individually use sticky medium containing 4 % (w/v) polyvinylpyrrolidone in TCM 199+ 10 % FBS with FSH suplementation then evaluated for their folicles development and oocyte quality. Result showed that the oocyte recovered from IVG after cultured in vitro for maturation resulted in lower rate of maturation of 0.09 % to 0.11 % But, IVG of preanthral follicle of local cattle could be accepted as an alternative of oocyte source for research in the near future.

Key words: Local cattle, Somatic cells, IVG. Cells banking

INTRODUCTION

The mammalian ovary contains a huge number of small follicle of various sizes, and each follicle enclose a small oocytes. In vitro growth (IVG) culturing of small oocytes will provide a new source of mature oocytes (M-II). Using the IVG culture system, non growing mammalian oocytes in primordial follicles possible grow to their final sizes and acquired full developmental competence. A small number of oocytes grow from minimal diameter size of 30 μm to the final size of 120 – 125 μm. Among large animals , it was reported that offsprings were produced from ovarian oocytes by IVG culture (Miyano, 2005).

The aims of this research are is to study the potential source of oocyte of Indonesian local Cattle of Madura, Peranakan Onggole (PO) and Bali using IVG method.If the small oocyte in the preantral follicles could be good cultured in vitro like normal oocyte growth, it will provide a new source of mature oocyte (M-II) for embryos production for local bovine. Recently, A very limeted number of a good quality of oocytes are available in our laboratory because of a little number female sloughtered locally. So far, the oocyte were isolated by apriration tehcnique of antral follicles. Meanwhile, In vitro growth (IVG) system could be improved as an alternative sources of oocyte both for research purposes as well as in vitro culture system.

MATERIAL AND METHOD

Cow ovaries were collected from local slaughter house. Preantral follicles that contain small growing oocytes are used as the material for IVG culture. Follicles with diameter of 2 – 3 mm was isolated and selected using micro-dissecting method (Miyano and Hirao, 2003). Preantral follicles were cultured for 14 days individually in 20 *u*l drops of sticky medium containing 4 % polyvinylpyrrolidone (Hirao et al. 2004) in TCM 199, supplemented with 10 % FBS heat activated, 10 % follicular fluid, 0.1 IU/ml Follicle Stimulating Hormon (FSH), pen strep, under paraffin oil in a humidified atmosphere of 5 % CO_2 in air at 37 ^{o}C. The follicles size cultured were classified in two group; small (< 4 mm and bigger (4.0 – 5.0 mm). Follicles were transferred to new medium drop every 48 hours. Evaluation was done under TV monitor (scaled and calibrated) connected with inverted microscope (Olympus).

RESULT DAN DISCUSSION

Preantrhal Follicle Recovered

The potential number follicles recovered from ovary of three of all three local cattle were 8.3 ± 3.95. It is considered as very large variation of follicle obtained per ovary might be of random sampling of bovine cattle isolated for their ovaries. Among these local animals, there are diffrent size of ovaries that following by different number of folicles recovered. These researches are mainly focusing on the developing the new potential of matured oocyte source.

In vitro growth of oocyte

Result of IVG culture showed in table 1. The IVG of preantral follicle of local cattle may could be accepted as an alternative of oocyte source for research in the near future base on the development oocyte capacity. Final size a part of of oocyte after IVG is reach about the same size approximately with matured oocyte, but it still need more information about proper mature of these cells. Preantrhar follicles total from 3 breed of diamater 0.2 – 0.3 mm were cultured for 2 weeks then evaluate of the diameter of oocyte and their morphology quality of cumulus-granulosa complex.

Table 1. Prelimenary result describes the potential of IVG result of the 3 Indonesian local cattle.

Cattle breed (number of ovary)	Follicle phase	Follicle obtained/ovaries.	Preantral Follicle size (mm).
Madura (33)	preantral	8.6 ± 2.35	0.2-0.35
Bali (27)	preantral	7.8 ± 4.9	0.2-0.45
PO (50)	preantral	8.7 ± 4.6	0.2-0.45

These result obtained may be not showed clearly yet the potential of IVG culture system as an alternative resources of matured oocyte because of limited data. But, these method is necessary to be developed for both potential animal livestock production and research in relation of providing more recipient oocyte, for example for nuclear transfer purposes. The ovary of cow contain a huge number of non-growing and growing oocytes. Aproximately 100.00 primordial follicle are contained in the cow ovary and 300 of them be developing to follicle (Gosden and Telfer, 1987; Miyano, 2006). A huge number of small oocytes are contained in the ovary of cow. A small number of them grow from the minimal size of 30 um in diameter to the final size of 120 – 125 um, then mature and are ovulated (Miyano and Hirao, 2003). A large number of the remaining oocytes do not enter the growth phase or degenerate in the ovary.). The potential number of these oocytes could be manage.

In general, cow oocyte grow from 30 μm to 120 – 125 μm to reach maturity, and a baby calf has been successfully produced from oocytes that had grown from 90 – 99 um in diameter. If these such small oocytes in the ovary could be better manage of growth, it will provide a new potential source of mature oocytes for recipient cell for nuclear transfer or animal in vitro fertilization programs. (Miyano, 2006). Bovine foillicle with diameter of 0.2 – 0.3 mm containe 70 -90 um oocyte, cultured in sticky medium of TCM 199 for 2 weeks and then evaluated for the oocytes. Almost all the oocyte recovered were enclosed by compact granulosa cells. The oocytes recovered were fuether cultured for maturation in the different treatment of FBS in TCM 199 stock. Result of IVM cultured shown in table 2.

Table 2. The quality of oocyte recovered following IVG, after IVM culture in TCM 199. Evaluation base on the expanded cumulus oocyte complex (coc). 2and 1 developed, expanded, 0 = not developed:

Treatment	Number of total oocytes	Oocyte Quality (%)		
		2	1	0
0 % FBS	180	5 (0.02)	85	90
5 % FBS	165	15 (0.09)	80	70
10 % FBS	152	17 (0.11)	80	55

The oocyte after cultured in vitro for maturation resulted in lower rate of maturation of 0.09 % to 0.11 % . Further reseach need to confirm these matured oocyte using IVF test for their competence. Early anthral folicle of cow has been reported (Yamamoto et al, 1999, Senbon and Miyano, 2002) their competence to mature in vitro, meanwhile report from Guiterez *et al*, (2000) dan Itoh *et al*, (2002) mentioned that oocyte resulted from IVG culture system not yel determined their maturation potential perfectly.

CONCLUSSION

It was concluded that In vitro growth of local cattle using small size oocyte will may provide potential source of matured oocyte in vitro for livestock production as well as for research purposes. The oocyte recovered from IVG after cultured in vitro for maturation resulted in lower rate of maturation of 0.09 % to 0.11 % It was suggested to do further research on IFV test of oocyte s resulted from IVG culture system.

ACKNOWLEDGMENTS

This research is a part of research funded by minister research and technology Republic of Indonesia.

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