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A Feasibility Study of Prepubertal and Over Mature Aged Local Goat in Relation to Results of *In Vitro* Growth Culture to Obtain Additional M-II Oocyte Resources

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Abstract. The aims of this research are to study the potential source of mature (M-II) oocytes of domestic animals using follicles isolated from prepubertal and over mature aged Indonesian local goats, resulting from an in vitro growth (IVG) method. This method of IVG could provide a new source of M-II oocytes for embryo production. In Indonesia, a very limited number of a good quality oocytes are available for research purposes, as there is a limited number of reproductive females slaughtered, which is dominated by prepubertal and old mature aged animals. IVG culture systems could be improved as an alternative method to provide a new source of a good quality oocytes for in vitro maturation of M-II oocytes. From a number of prepubertal and mature aged goats slaughtered in a local abattoir, the small oocytes in the preantral follicles were cultured in vitro to normal oocyte growth. The methods used in this research are experimental. Follicles were isolated, cultured in vitro for 14 days individually using a sticky medium containing 4% (w/v) polyvinylpyrrolidone in TCM 199 10% Fetal Bovine Serum supplemented with Follicle Stimulating Hormone, which was then evaluated for their follicle development and oocyte quality. The research results showed that a minimum follicle size and oocyte diameter is needed (>100 um) for early evaluation of maturation to be achieved, meanwhile oocytes recovered from IVG after being cultured in vitro for maturation resulted in a very low rate of maturation. However, in the future, IVG of the preantral follicles of Indonesian local goat could be considered as an alternative source of oocytes for both research purposes and embryo production in vitro.

INTRODUCTION

The animal ovary contains a huge number of small follicles of various sizes and shapes,with each follicle enclosing a small immature oocyte. In vitro growth (IVG) culturing of preantral follicles and small oocytes will provide a new source of mature oocytes (M-II). In the field of livestock production, IVG of small ovarian oocytes will provide a large number of mature oocytes. Using an IVG culture system, non-growing mammalian oocytes in primordial follicles could possibly grow to their final sizes and acquire full developmental competence. However, the IVG system for domestic species, in which follicles developed to a much larger size requires a longer period of time, has to be established especially for local goats. A small number of oocytes grow from a minimum diameter size of 30 µm to the final size of 120–125 µm. Among large animals, it has been reported that offspring were produced from ovarian oocytes by IVG culture [1]. Porcine and bovine oocytes do not complete their growth in the early antral follicle until they develop into the late antral stage with a diameter of about 5 mm [2]. The follicle size and stage in which oocyte growth is completed differ among species. The local goats slaughtered at this area of Malang, Indonesia were dominated by a younger female or prepubertal animals and old mature aged goats.

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The aims of this research are to study the potential source of an oocyte of Indonesian local domestic animals, namely the Etawah crossing grade goat, of prepubertal and old mature aged goats using an IVG method. If the small oocyte in the preantral follicles could be well cultured in vitro, similar to normal oocyte growth in vivo, it will provide a new source of M-II oocytes for embryo production. Recently, a very limited number of a good quality oocytes became available in our laboratory in Indonesia because of a small number of females slaughtered locally, which were dominated by prepubertal and old mature aged goats. So far for research purposes, the oocytes were isolated by aspiration technique of the antral follicles. Meanwhile, IVG systems for culturing preantral follicles and growing small sized oocytes inside could be improved as an alternative source of oocytes both for research purposes, as well as in vitro culture system research and development.

EXPERIMENTAL DETAILS

The goat ovaries were collected from a local slaughterhouse in Malang city. Preantral follicles containing small growing oocytes were used as the material for IVG culture. Follicles with the diameter of 2.0–3.0 mm were isolated and selected using a micro-dissecting method [3]. Preantral follicles of different groups of aged (prepubertal and old mature aged) goat were cultured individually for 14 days in 20 ul drops of sticky medium containing 4% polyvinylpyrrolidone [4] in TCM 199, supplemented with 10% heat activated Fetal Bovine Serum (FBS), 10% follicular fluid, 0.1 IU/ml Follicle Stimulating Hormone (FSH), pen-strep, under paraffin oil in a humidified atmosphere of 5% CO_2 in air at 37 ^{o}C.

The follicle sizes cultured were classified and selected for a small category (2.0–3.0 mm of diameter). Evaluation of follicle development was done under TV monitor (scaled and calibrated) connected to an inverted microscope (Olympus). The variables observed included the number follicles obtained per ovary, morphology and size of an ovary, oocyte diameter size and quality of oocytes before and after IVG (µm). Data were analyzed descriptively for morphological and t-test analysis for a comparison before and after IVG culture.

RESULT AND DISCUSSION

Preantral Follicle Recovered

The potential of preantral follicles isolated from the local goat is relatively very low (TABLE 1). The follicle sizes recovered from the ovaries of goats is considered as having a large variation in follicle number obtained per ovary, which might be the resultof random sampling and the limited number of goats slaughtered and isolated from their ovaries. Among these local goats, there are different sizes of ovaries with different numbers of follicles being recovered.

TABLE 1. Preliminary result describes the potential of IVG result of the Indonesian local goat of PE breed in alocal slaughtered house in Malang.

No	Group of ages and number of the ovary (n).	Follicle phase	Follicle obtained/ovaries.
1	Prepubertal Goat (80)	Pre antral	5.6 $\pm$ 2.3
2	Over Matured Goat (78)	Pre antral	11.4 $\pm$ 3.2

These results, based on the follicles obtained (TABLE 2) may clearly demonstrate the potential of IVG culture systems as an alternative source of matured oocytes because of limited data. However, this method is necessary to develop for both potential animal livestock production and research in relation to providing more recipient oocytes, for example for nuclear transfer purposes. The ovary of the cow contains a huge number of non-growing and growing oocytes. Approximately 10–000 primordial follicles are contained in the cow ovary, with 300 of them developing to follicles [1,5]. A huge number of small oocytes are contained in the ovary of a cow. A small number of them grow from the minimal size of 30 um in diameter to the final size of 120–125 µm, then mature and are ovulated [3]. A large number of the remaining oocytes do not enter the growth phase or degenerate in the ovary. The potential number of these local goat oocytes could be managed.

In general, oocyte grows ranges from 30 µm to 120–125 µm to reach maturity, and a baby calf has been successfully produced from oocytes grown from 90–99 µm in diameter. If such small oocytes in the ovary could be

better managed for growth, they will provide a new potential source of mature oocytes for recipient cells for nuclear transfer or animal in vitro fertilization programs [1]. Bovine follicles with a diameter of 0.2–0.3 mm containing 70–90 µm diameter oocytes, have been cultured in the sticky medium of TCM 199 for two weeks and then evaluated for oocytes. Almost all the oocytes recovered were enclosed by compact granulosa cells. The oocytes recovered were further cultured for maturation in the different treatment of FBS in TCM 199 stock.

A comparison of two different age level (prepubertal and old mature aged) goat showed that there are important characteristic differences between prepubertal and older goats (**TABLE 2**).

TABLE 2. Different character morphometric of theovary of different level of age of local goat.

No	Parameters	Prepubertal age	Over matured age of goat
1.	Length (mm)	13.14 ± 2.40	20.66 ± 2.40
2.	Shape (mm)	9.14 ± 1.90	13.80 ± 2.70
3.	Ovary volume size (mm^2)	97.56 ± 32.40	226.49 ± 56.80

Oocyte Growth and Quality after IVG

A comparison between oocytes before and after IVG are presented in **TABLE 3**. IVG of the preantral follicle of the local goat could be considered as an alternative oocyte source for research in the near future based on the developing oocyte capacity. The final size of the oocytes after IVG reaches about the same size approximately as the matured oocyte, but further information about the proper maturity of these cells is required. Preantral follicles from goats of diameter 0.2–0.3 mm were cultured for 2 weeks, then evaluated for the diameter of oocytes and their morphological quality of cumulus-granulosa complex.

TABLE 3. The morphological character of ovary and oocyte development and quality resulted from IVG.

No	Parameters	Prepuberty	Aged of age
1.	Qualiy of oocyte base on the expanded level of Cumulus oocyte complex	0.82 ± 0.80 [a]	1.47 ± 0.6 [a]
2.	The average final size of oocyte after IVG (µm)	120.26 ± 33.00[a]	128.07 ± 33.58[b]
3.	Oocyte growth rate (µm)		
	Prepubertal:before and after 14 days culture IVG	54.24 ± 8.10[a]	134.08 ± 8.7[b]
	Old Matured: before and after 14 days of IVG culture	60.9 ± 9.0[a]	149.94± 12.50[b]

*Different subscript in the same row mean very significant different (P≤ 0.01). The expanded cumulus-oocyte complex (coc) : 2 and 1 = developed, expanded, 0 = not developed:

The oocytes after culture in vitro for maturation resulted in a lower rate of maturation, based on the expandedcumulus-oocyte complex. Further research needs to confirm these matured oocytes using an IVF test of their competence. Early antral follicles of cows have been investigated regarding their competence to mature in vitro[6], meanwhile reports from Guiterez et al. [7] mentioned that oocytes resulting from IVG culture systems had not yet determined their maturation potential perfectly.

The result regarding IVG from this research is relatively low and faces a significant problem regarding contamination. The culture system requires considerable improvement to increase its efficiency, especially when using large species of domestic animals whose oocytes take a much longer time to reach their final size. Miyano[1] mentioned that maintenance of the viability of the oocyte and the surrounding granulosa cells is a major problem. Porcine and bovine oocytes grow to a volume 3.5–5.0 times larger than those of mouse oocytes. In bovine IVG culture systems, supplementation with a meiotic arresting substance such as hypoxanthine is essential. In this culture system, we used supplementation of follicular fluid in a fixed presentation (5%) rather than hypoxanthine. Down et al. [9] reported that hypoxanthine in follicular fluid has been identified as one such meiosis arresting substance.

For the near future, the potential of IVG culture systems for oocytes is expected to provide a new source of a large population of M-II oocytes. The results reported regarding several species is relatively promising both for research purposes as well for embryo production in vitro (**TABLE 3**). For future research, we need to confirm the potential of M-II oocytes through calcium dynamics during maturation of goat oocytes after 24 hours of culture

using confocal laser scanning (CLSM-fluo-3). We analyzed the calcium intensity base on the histogram profile of oocyte and spermatozoa considered. There is quite a variation in the oocyte calcium intensity.[9]

Results reported from IVG culture systems from different species of the mammalian oocyte [1,10], from 1989 to 2015 show a different potential of different species including mouse, pig, and cow with matrix collagen, resulting in a positive result for IVM or IFV methods and obtaining offspring. In domestic species, the first successful IVG culture system that supports the growth of oocytes from mid-growth phase in preantral (late secondary) follicles to the final size was reported by Hirao et al. [11] (Table 3). It has been reported that sheep preantral follicles grow to the antral stage in serum-free conditions after one month of culture and a small number of in vitro grown oocytes mature to metaphase (M-II) [12].

SUMMARY

IVG of Indonesian, local goat using preantral follicles and small size oocytes will provide a potential source of M-II oocytes in vitro for both research development and livestock production purposes. The follicle and oocyte size is an important parameter to consider in the early evaluation of their quality. The results showed that follicle sizes are significantly different between two groups (prepubertal and mature aged) of agoat. The number of follicles obtained from the two age groupswas not different. The minimum follicle size and oocyte diameter needed (>100 μm) for early evaluation of maturation were achieved. The final size of oocyte after IVG reaches about the same size approximately as a matured oocyte. Meanwhile, oocytes recovered from IVG after culture in vitro for maturation resulted in a very low rate of maturation to M-II. However, in the near future, IVG of the preantral follicle of Indonesian local goat could be considered as an alternative source of oocytes for both research purposes and embryo production. The oocytes recovered from IVG after culture in vitro for maturation resulted in a very low rate of maturation (IVM). Further research is suggested on IFV testing of oocytes resulting from IVG culture systems.

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REFERENCES

1. T. Miyano, J. of Reproduction and Development **51**, 169 – 176 (2005).
2. Y. Hirao, Y. Tsuji, T. Miyano, A. Okano, M. Miyake, S. Sato and R. M. Noor, Zygote **3**, 325 – 332 (1995).
3. T. Miyano and Y. Hirao, J. Mammal Ova Research **20**, 78 – 85 (2003).
4. Y. Hirao, T. Itoh, M. Shimizu, K. Iga, K. Aoyagi, M. Kobayashi, M. Kacchi, H. Hoshi and N. Takenouchi, J. BiolReprod **70**, 83-91 (2004).
5. R. G. Gosden and E. E. Telfer, J. Zool, London **211**, 169 – 175 (1987).
6. S. Senbon and T. Miyano, Zygote **10**, 301 – 309 (2002).
7. C. G. Gutierrez, J. H. Ralhp, E. E. Teffer, I. Wilmut and R. Webb, Biol Reprod. **62**, 1322 – 1328 (2000).
8. S. M. Downs, D. I. Colemen, P. F. Ward-Bailey and J. J. Eppig, Proc. Nat Acad Scie. USA **82**, 454 – 458 (2004).
9. A. A. G. Adam, Y. Takahashi, S. Katagiri and M Nagano, J. of Reproduction and Development **50**, 579 – 586 (2004).
10. Y. Hirao, Y. Tsuji, T. Miyano, A. Okano, M. Miyake, S. Sato S and R. M. Noor, Zygote **3**, 325 – 332 (1995).
11. S. Cecconi, B. Barboni B, M. Coccia and M. Mattioli, J. Biol Reprod **60**, 594 – 601 (1999).
12. A. Hasegawa, A. N. Mochida and H. Kasumi, J. Mammam. Ova Research. 24: 8 – 12 (2004)