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Abstract. These study purposes are to analyse the potential and benefits of utilization local goat ovary waste obtained at slaughterhouses as a source of cells or genetic material for the purposes of research, conservation, and freezing of genetic material of immature and mature oocyte in vitro. Preantral and antral follicles were selected and isolated for an experimental In Vitro Maturation (IVM) and Growth (IVG) of immature small size oocytes and goat antral and preantral follicles. The research method is explorative using a case research about the potential supply of follicle cells and immature oocytes sources of a local goat. Ovaries obtained from selected goat from the slaughterhouse are isolated for follicles and immature oocytes for culture in vitro and cell freezing. Data obtained are presented in the mean value and standard deviation and analyzed descriptively. Each ovary for obtaining follicle and immature oocyte was selected and isolated using 2 different methods of slicing and aspiration. The result showed that the great potential of the material genetic source of local goat from the different condition of goat slaughtered in the different potential of the follicle (IVG) and immature oocyte isolated (IVM) for each ovary (3 to 6 follicles) and (4 to 8 oocytes) respectively. The result is considered as a high variation because of different condition and variation of ages from prepubertal goat to adult goat of more 3 years of ages. A number of the follicle and immature oocytes may be isolated of about 200 per day. The IVG of follicle resulted in a lower result of M-II.oocytes of about 10 % oocyte recovered, meanwhile IVM of immature small oocyte resulted of about 40 to 60 % maturation (IVM) base on the cumulus expansion and first polar body extrusion. Culture system IVG may provide not only of how producing a number of competence mature oocytes but also of investigating the physiology of follicular development and ovulation of Indonesian local goat, it 's may produce many matured oocytes (M-II) in the possibility of high numbers for research development and genetic material conservation. It was concluded that both IVG and IVM methods may possible to utilize ovary waste as an important source of genetic material for research development cell culture and cell conservation.

1. Introduction

One of the main problem related to research and development in reproductive biotechnology, especially in cell culture in vitro, is the availability of gamete cells both for sperm and oocyte. So far, In vitro

research in Indonesia is often faced a limited number of oocytes in term of both quantity and quality. In general, female gamete cells or oocytes were obtained from animals slaughtered in a slaughterhouse.

By-products from animal slaughterhouses, especially those in the form of reproductive organs and accessories so far have not been used properly. In fact, the ovary is one of the potential wastes in which there are follicles or oocytes that can be used to research the development and application of reproductive biotechnology and conservation and cell freezing. This is generally caused the availability of genetic material in the cell is very low, by the great varied quality of livestock, the high variation age of livestock animals and the number of animals slaughtered in slaughterhouses is very limited.

The goat and others livestock animals ovary contain an important large number calculated of small follicles of various conditions, sizes, morphology, and shapes, with each of follicle enclosing the small immature goat oocyte. The in vitro growth culture of preantral follicles and IVM small oocytes will possible to provide a new potentials source of mature oocytes (M-II). The IVG can be used to support IVM, which has been implemented earlier in many cell culture laboratories [1, 12]. In the field of livestock assisted reproductive technologies, IVG of small ovarian and oocytes will provide a large number of mature oocytes, nongrowing oocytes cells in primordial follicles could possibly develop and grow to acquire cells full developmental competence. However, the IVG culture system for the domestic and livestock animals, in which prefollicles developed to a much larger size requires a longer period of time than normal periods, has to be established, especially for local goats with may be a spesific condition in Indonesia. It was considered that for ruminant in normal condition, a small number of oocytes cells could grow from a minimum diameter size of about 30 μm to the final size between 120 to125 μm. Among large livestock animals, it has been reported that offsprings were born of ovarian oocytes by in vitro growth culture [1]. Porcine, bovine and other female ruminants cell oocytes could not complete their development and growth in the early stage of antral follicles until they develop into the late antral phase with a diameter size of about 5 mm [2, 15]. Theorytically, it is understood that the follicle size, phase or stage in which oocyte cells growth and develop, is completed differ among species. Information and data about IVG and IVM potential in local goat follicles and oocytes are still very limited.

The local PE goat selected and slaughtered at around area of Malang city, Indonesia was mainly dominated by a younger age female or prepubertal animals. A number of local goats slaughtered in Malang slaughterhouses of about 20 to 50 heads per day with dominated by more than 85 % of a female goat at younger ages [13], and in certain days it could achieve more than 150 heads per days [11, 12]. If, it assumed that an average of 4 to 6 antral follicles could be isolated, about more than 200 oocytes can be recovered per day for research and development purposes.

The goals of this research are to estimate and to study the real potential and its utilization source of an oocyte of Indonesian local domestic animals, namely PE goat or the Etawah crossing grade using IVM and IVG methods, it may provide a new source of M-II oocytes for research biological science development or even for commercial embryo production. At this time, a very limited number of good quality oocytes may become available in our cells culture laboratory because of a very small number and limited good quality of females goat slaughtered locally, which were mainly dominated by prepubertal and old mature or over aged goats which are not in the optimal reproductive period. Therefore, for these research, the valuable oocytes were isolated by aspiration technique of the antral follicles. Meanwhile, IVG culture systems for developing preantral follicles and growing small sized oocytes could be improved as an alternative source of matured (Metaphase-II) oocytes for research purposes, as well as in vitro culture system, development and as a model for understanding physiology biology of cell development in vitro.

2. Materials and methods

The reproductive organ part of local goat with ovaries was collected as soon as possible after animals were slaughtered, and isolated from a local slaughterhouse owned by local government in Malang city. The local goat used are Indonesian specific local breed goats, namely Etawah Grade (PE) with variations in an age less than one year to more than three years. Goats are grouped into 4 age of groups that is,

sequentially less than 1 year age, 1 to 2 years age, 2 to 3 years and over 3 years of ages model for understanding physiology biology of cell development in vitro.

Preantral follicles isolation results that are still intact and considered containing small growing oocytes, were used as the material for IVG culture. The selected ovary was taken to the laboratory in physiological NaCl in a sterile bottle, and put in a thermos of warm water 30 ℃ at the latest 3 hours after the animal was slaughtered. In the laboratory, ovaries were cleaned from debris, adhering fat tissue and washing 3 times in physiological NaCl. At first, the ovaries are used for IVM, by isolating the oocytes in the antral follicles, then the rest is sliced or follicular preantral isolation for IVG. In case of younger animal ovary without antral follicles were directly isolated their preantral follicle by slicing method [15].

The preantral goat follicles were collected with slicing technique, which one has the diameter size of 2.0 to 3.0 mm were isolated and selected using a micro-dissecting standard method [3]. This follicle isolation technique with slicing has a great chance that the follicle can lysis because it has not mastered the protocol and the skill is lacking [14, 15].

Preantral follicles of different goat slaughtered groups with different age (prepubertal, pubertal and old mature aged) were cultured individually for 14 days successively in 20 ul each drops of sticky medium containing of 4% polyvinylpyrrolidone supplementation [4] in TCM 199 medium, supplemented with 10% heat activated Fetal Bovine Serum (FBS), 10% follicular fluid, 0.1 IU/ml FSH, antibiotics pen-strep, covered under sterilized paraffin oil, in a humidified atmosphere of 5% CO2 in air at 37 ℃ incubator. The preantral follicle sizes cultured for IVG are classified and selected for onlt the small category (2.0 to 3.0 mm of diameter size).

The evaluation of preantral follicle development was performed in the fixed time of culture under television monitor equipment (which equipment scaled and calibrated have been done) and it was connected to a sophisticated inverted microscope (Olympus). The parameters observed in this research were included the number of goats slaughtered/day, number of ovaries obtained/day, number of preantral and antral follicle isolated/animal, follicles obtained/ovary, condition of ovary, morphology, quantitative characters and sizes of an ovary, oocyte obtained/ovary, oocyte diameter (μm) size and quality of oocytes before and after IVG. The qualitative data obtained were analyzed descriptively for their morphological and a quantitative t-test analysis was performed for a biological data comparison before and after IVG cell culture results.

3. Result and discussion

In general, a number of goats slaughtered in Malang City, East Java, especially in official slaughterhouse tend to decrease in this 5 recent years. Goat slaughtered were dominated by a younger female goat (Table 1).

Table 1. Identification of the potentials goat slaughtered in Malang city base on the sex and ages during the low season, one month observations.

Item	Character	Goat sloughtered/day (heads)	Percentage (%)
Sex	Male	28	15.09
	Female	148	84.09
Ages	Less than 1 year	66	46.47
	Between 1 - 2 years	34	23.94
	Between 2-3 years	18	12.67
	More than 3 years	24	16.90

Animals slaughtered were dominated by female and younger old which considered not yet reached puberty of optimum reproductive potentials (46.47 %). These animals in this condition require a special attention because of the low quality of follicles and oocyte in preparation for maturation (M-II oocyte).

At this age condition in vitro cells need hormonal supplementation to stimulate follicular growth from exterior source.

The potential of preantral animal follicles, as well as immature oocytes isolated from the local goat, is relatively categorized very low. Meanwhile, recent data of potential antral follicle was also limited in both numbers of antral and preantral follicle as well as their quality (Table 2). Both antral and preantral follicle sizes recovered from the ovaries of local goats is considered as having a large variation in follicle size and number obtained per ovary. Many technical factors and it's also related to the material used have an effect on the developed structural system, including the main ones which might be because of random and total sampling and the limited number of local goats slaughtered per day and isolated from their ovaries. Practically no rigorous selection of animals has been carried out, because of animal samples are very limited, so almost all of the goat female slaughtered are used as an ovary source. After all, among these local goats, we observed different sizes of ovaries with different numbers of follicles being recovered. This condition may be reflected in the lower IVG and IVM success rate.

Table 2. Early result describes the potential of IVG by follicle obtained/ovary of the Indonesian a local animal of PE goat breed in local slaughtered house in Malang city [15, modified].

No.	Group of ages	Follicle phase	Number Follicle obtained/ovaries (X+SD)
1	Prepubertal local goat category	Pre antral (slicing method)	4.60 + 1.40
2	Over Matured local Goat	Pre antral (slicing method)	10.40 + 2.20
3	Pre pubertal and pubertas local goat	Anthral folicle (aspirating method)	7.03 + 2.49

Based on the initial results of this research, mainly based on the follicles obtained per ovaries may clearly demonstrate and provide temporary evidence that the low potential of IVG culture systems as categorize as low as an alternative source of matured oocytes (Metaphase-II) because of limited data. However, this method is necessary to be developed for both potential animal livestock production for next near future, reproduction and research in relation to providing more recipient oocytes (M-II) available, for example for biological research in nuclear transfer purposes as a tool of reproductive biotechnology which continues to grow rapidly [11]. The ovary of ruminant i.e. cow and others ruminants contains an important number of non-growing and growing oocytes. Approximately an important number of 10–000 primordial follicles are contained in the cow ovary, with 300 of them has the potential to develope to normal antral follicles [5, 9]. An important and 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 to 125 μm, then mature and are ovulated [3]. A large number of the remaining oocytes animals do not enter to the growth phase or will be degenerate in the ovary. There is a correlation between follicles that grow in size and the quality of oocytes inside of developed follicle [15].

Actually, the potential source of oocyte cell number of these local goat oocytes could be managed for improving their quality. In general, animal's oocyte of bovine grows ranges from 30 μm to 120 to125 μm to reach maturity, and an offspring calf has been successfully produced from oocytes grown from 90 to 99 μm in diameter. With a culture method in vitro for cells growth, it will provide a new potential source of mature oocytes for recipient cells i.e. for nuclear transfer or animal in vitro fertilization programs [1]. Bovine follicles with a diameter of 0.2 to 0.3 mm containing 70 to 90 μm diameter oocytes, have been cultured in the sticky medium of TCM-199 medium for two weeks and then evaluated for oocytes growing and quality. The oocytes recovered were further cultured for maturation in the different treatment of FBS in TCM- 199 stock.

Different age level as categorized as prepubertal and over mature aged goat showed that there are important characteristic differences between prepubertal and older goats (Table 2). The main problem

in IVG culture is the occurrence of very high contamination which is also driven by the long duration of cell culture. Both goat age groups showed the ability to develop into its optimum size as a mature of the oocyte's competency.

Oocyte growth and quality of oocyte after IVG showed the significant different of oocyte size, contrary to the oocyte quality base on the expanded cumulus. A comparison between oocytes before and after IVG are presented in Table 3. The 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, but this method is considered necessary to be developed and improving the success rate. 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. The preantral follicles from goats with a diameter of 0.2 to 0.3 mm were cultured for two weeks, then evaluated for the diameter of oocytes and their morphological quality of cumulus-granulosa complex.

The research results reported a significant correlation between follicle size and oocyte diameter and between a diameter of immature oocytes with COCs quality [11], Meanwhile, culture system using MSOF medium with gonadotrophin supplementation on maturation rate of an immature oocyte of goat in vitro have no significant difference in maturation rate [12]. Goat oocyte culture with IVM requires a longer time, if in general cattle find maturity of cells at 24 hours, then goats need 26 to 28 hours of IVM culture [14].

Table 3. The morphological characters observed of ovary and oocyte of local two level goat ages on the development and quality resulted from IVG, base on the expanded cumulus and final size of oocytes [15, modified].

No	Parameters observed	Prepubertal (less than 1 year age) (X±SD)	Over matured age of goat (more than 3 years age (X±SD)
1	Quality of oocyte base on the expanded level of Cumulus oocyte complex	0.82 ± 0.80^{a}	1.47 ± 0.6^{a}
2	Final size of oocyte after IVG (*u*m)	120.26 ± 33.00^{a}	128.07 ± 33.58^{b}

The expanded cumulus-oocyte complex (CoC): 2 and 1 as categorized as developed, expanded, but 0 is categorized as not developed cell. The oocytes after culture in vitro for maturation with TCM 199 medium, resulted in a lower rate of maturation, based on the expanded cumulus-oocyte complex. Further research needs to confirm these matured oocytes using an IVF test of their competence to be fertilized by sperms. The important thing that must be understood is the success of the IVG technique that is able to produce M-II oocytes which will complement the number of M-II oocytes that can only be obtained through IVM and ovum pick-up. Early antral follicles of cows have been investigated regarding their competence to mature in vitro, meanwhile reports from [6] 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 factor that influences IVG's success rate in this research is a long culture that is long enough and requires regular medium replacement techniques so that human and environmental factors are very influential. 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. The result from [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 to5.0 times larger than those of mouse oocytes.

In this culture system, we used supplementation of follicular fluid of goat in a fixed presentation (5%) rather than hypoxanthine with the main medium culture of TCM199. The result of [7] reported

that the hypoxanthine in follicular fluid has been identified as one such as meiosis arresting substance. For the near future, the potential of IVG culture systems for oocytes are expected to provide a new source of a large population of Metaphase-II oocytes. For local goats, especially younger goat its is considered very potential because at this age ovary does not have an antral follicle which allows its oocytes to be aspirated so that surgical techniques for follicular isolation can be performed.

The results reported regarding several species is relatively promising both for research purposes as well for embryo production in vitro [11,12,14,15]. For future research and development, we need to perform a advanced study to confirm the potential of M-II oocytes through calcium dynamics during maturation of goat oocytes after 24 hours of culture [14] using confocal laser scanning microscope (CLSM-with fluo-3 stainning). The analyzed of the calcium intensity base on the histogram profile of oocyte non and matured, as well as to indentify live-dead cells and maturation rate supporting with result reported before [15]. It was reported quite important variation in the oocyte calcium intensity, there are significant different profile of immatured and matured calcium band intensity [7].

In general, many results reported from IVG culture systems from different species of the animals and mammalian oocyte [1,8], show a different potential of different species, resulting in a positive result for IVM or IFV methods and finally obtaining offspring. In domestic species, the first successful IVG culture system that supports the growth of oocytes from mid-growth phase in preantral follicles to the final size was reported by [9].

This potential supply and availability of immature small oocyte cells and also ruminant follicles will be greatly increased if we can utilize a large number of other slaughterhouses in Indonesia, especially in bigger cities and goat production centers. Besides goats, actually, some other animal species such as sheep and cattle can be used, so that in Indonesia it can have a kind of local livestock cell bank in the form of oocytes. 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) [10].

In Indonesia local goats, it seems that there is still a need to study later about the optimization of the culture system for both IVM and IVG so that better success rate can be obtained. The quality factor of ovaries obtained from animal slaughterhouses is an important thing to consider.

4. Conclusions

The in vitro culture cell method both of IVG and IVM of Indonesian local goat using preantral follicles and small size oocytes or immature oocytes may provide a potential source of mature (M-II) oocytes in vitro for both research development and livestock reproductive purposes. In the near future, IVG of the preantral follicle as well as antral follicle for IVM of differents age level of Indonesian local goat could be considered as an alternative source of oocytes for both research purposes and embryo production in vitro. The oocytes recovered from IVG after performing culture in vitro for maturation resulted in a low rate of maturation. The IVG technique optimization for local goats in Indonesia still needs to be further developed in order to obtain better results. Further detail research is recommended on fertilization in vitro (FIV) test of oocytes competence result from in vitro growth culture systems with preantral follicles.

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