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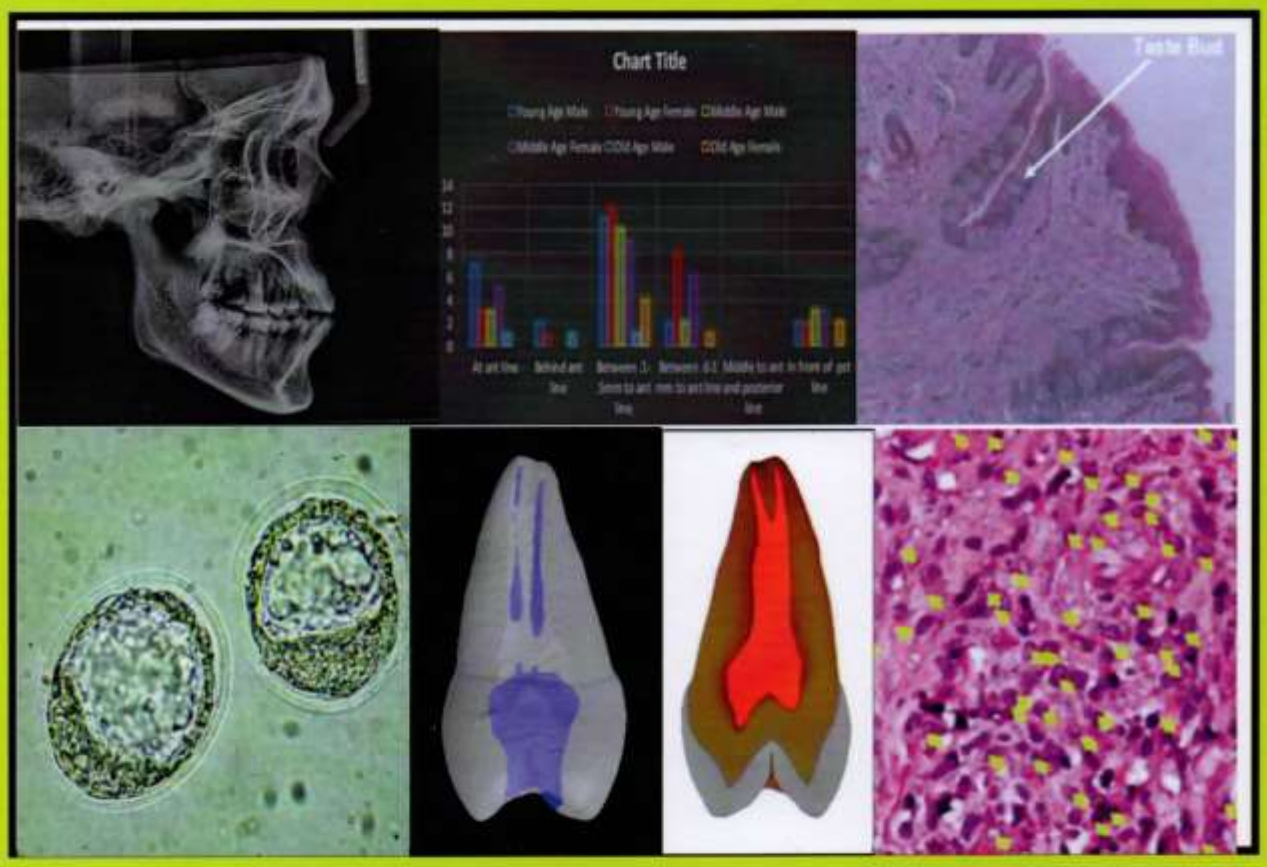

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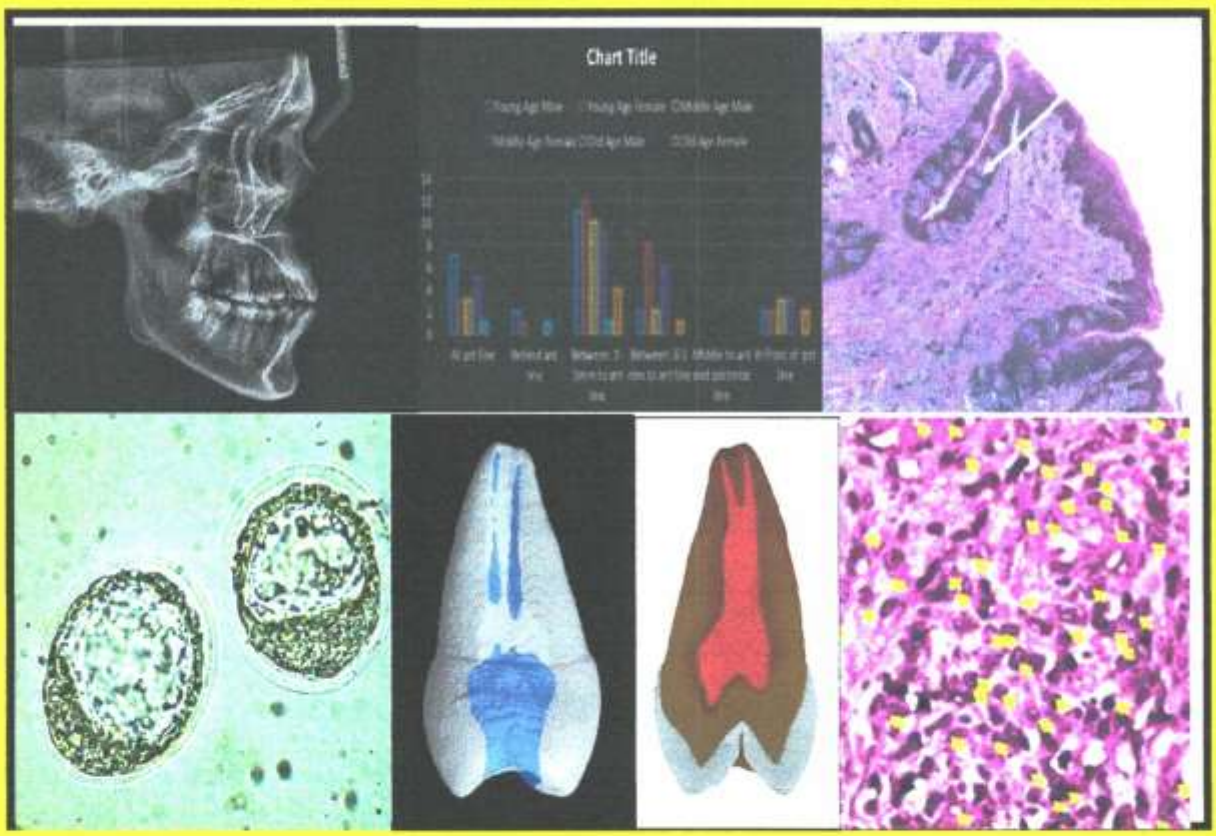

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Maturity and Apoptosis Rate of Cumulus - Oocyte Complex in Aceh Cattle after in Vitro Maturation

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Abstract

The study aims to find out maturity dan apoptosisrate of cumulus -oocyte complex in aceh cattle after in vitro maturation (IVF). The oocytes used in this study amounted to 95 oocytes from ovary waste taken from slaughterhouse. The oocytes were collected by aspiration method. All oocytes were maturated in DMEM medium at 38°C 5% CO2 for 20 hours. After maturation, maturity and apoptosis rate of cumulus -oocyte complexwas calculated.

The results showed that the maturity rate of aceh cattle oocytes reached 65.71% and the apoptosis rate reached 13.3%. Based on the results of this study it can be concluded that the oocytes of aceh cattle have good maturity rate and low apoptosis rate ofcumulus - oocytecomplex after in vitro maturation.

Experimental article (J Int Dent Med Res 2017; 10(3): pp. 1066-1069)

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Introduction

Development of aceh cattle as one of genetic resources of beef cattle has been attempted. Various introduction on reproductive technology on aceh cattle keeps being applied in order to obtain maximum productivity and good reproduction. Aceh cattle is one of genetic resources with its population kept and maintained to increase food availability in Indonesia particularly in Aceh Province. Some researchers have applied reproductive technology on aceh cattle, however, the result is not satisfactory[1]. Reproductive technology has been much applied on livestock such as cattle. The technology covers in vitro fertilization, in vitro embryo production, embryo transfer, ICSI (*i*ntracytoplasmic sperm injection), and cloning. However, the practice to help aceh cattle development is not maximum yet.

Development of rapid reproductive technology shows that processes that take place inside animal body is able to be done outside animal body which is known as in vitro. In vitro oocyte maturation is conducted in order that primary oocyte is able to complete meiosis process so that it will develop into secondary oocyte which is haploid and has ability to be successfully fertilized by spermatozoa and is able to support next embryo development. During ovulation, oocyte is at break stage of metaphase II until oocyte activation takes place to continue development[2,3]. Maternal mRNA accumulation and molecular and structural change such as increase of number and size of organelle take place during cytoplasm maturity. Change on the cell organelle is able to make oocyte have ability to support fertilization process and embryo development[4]. Until now, research on in vitro oocyte maturation process and evaluation on oocyte apoptosis after in vitro maturation on aceh cattle have not been conducted yet.

The study aims to find out aceh cattle oocyte rate after in vitro maturation and evaluate apoptosis of maturatedoocyte. The result of the study is hoped to be able to make use of reproductive technology to help development of aceh cattle population particularly in Aceh Province.

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Materials and methods

Aceh cattle oocyte collection

The study used six pairs of ovaries of grown aceh cattle. The ovaries were obtained from slaughterhouse. The ovaries were washed with physiological NaCl 0.95% supplemented with gentamycin 0.5 ml and oocyte collection process was then carried out. Oocyte collection was done by aspiration using spuit with needle 18G containing solution of *p*hosphate buffer saline(PBS) 5 ml. Collected Oocytes were put in sterile petri dish and then continued with observation under microscope to observe oocytes used. Oocytes used had normal morphology with characteristics of homogeneous cytoplasm, whole membrane, and surrounded by cumulus cells.

There were eight aceh cattle oocytes successfully collected. Collected oocytes were washed three times in solution of PBS and then washed using maturation medium of DMEM
(*D*ulbecco's modified Eagle's medium) three times.

In vitro oocyte maturation

Cleaned oocytes were moved to DMEM medium as maturation medium for maturation process which was covered with mineral oil (Sigma, USA). Maturation process was done in CO_2incubator5% at the temperature of 38ºC for twenty hours until cumulus cells expansion took place.

Examination of cumulus oocyte complex maturity rate

Examination of cumulus oocyte complex maturity rate was conducted microscopically by counting number of mature oocytes based on expansion rate of cumulus cells. Mature oocytes were marked by loose cumulus cells and hyaluronic acid secretion that seems to have expansion. Data obtained were tabulated and percentage was counted.

Examination of cumulus oocyte complex apoptosisusing immunocytochemistry

Examination on apoptosis was conducted using immunocytochemistry method by using antibody to cells that experiencedapoptosis. Mature oocytes from in vitro process were put in object glass and fixated using fixation solution of mixed metanol andacetic acid glacial (1:3). Fixated oocytes were destained using tunnel method.

Results

Aceh cattle oocytes which were successfully collected amounted to 95 oocytes which met requirement for in vitro maturation. Collected oocytes were then maturated in DMEM medium. Result on in vitro maturation of aceh cattle oocytes is shown in Table 1, Figure 1 and Figure 2.

Oocyte number	Oocyte number (percentage)	
	Experience maturation	Do not experience maturation
95 oocytes	68 oocytes (71.58%)	27 oocytes (28.42%)

Table 1. Maturity rate of aceh cattle oocytes after 20 hour in vitro maturation.

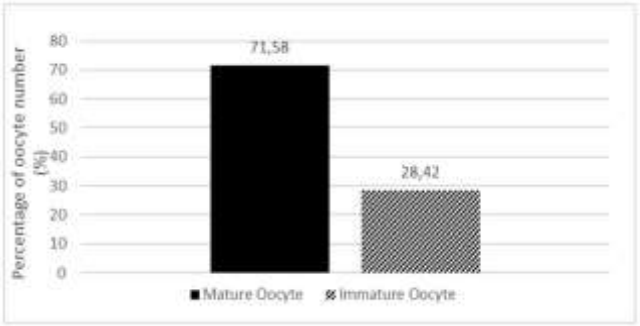

Figure 1. Percentage of aceh cattle oocytes after in vitro maturation for 22 hours.

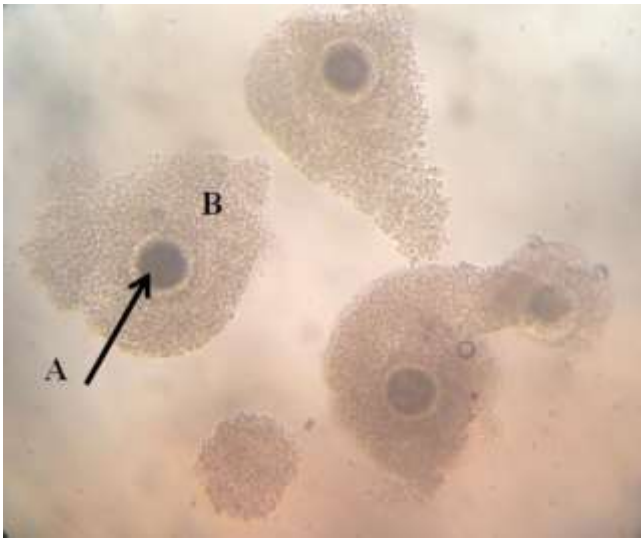

Figure 2. Aceh cattle oocytes after in vitro maturation for 20 hours. The figure shows that cumulus cells spread equally to surround oocytes.
A : oocytes, B : cumulus cells.

Based on Table 1 and Figure 1, it is known that aceh cattle oocytes were able to experience in vitro maturation with the percentage of 65.71%. Oocytes after maturation were marked by spread of loose cumulus oophorus which surrounded oocytes and had light color (Figure 2).

Oocyte number after in vitro maturation	Observation (%)	
	Apoptosis	No Apoptosis
30	4 (13.33%)	26 (86.67%)

Table 2. Apoptosis examination on cumulus oocyte complex after in vitro maturation.

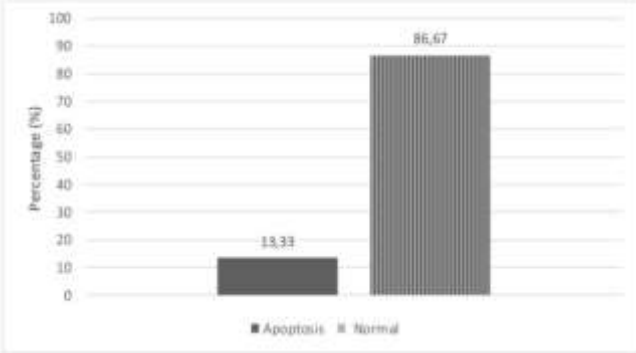

Figure3. Percentage of apoptosis on aceh cattle cumulus oocyte complex after in vitro maturation.

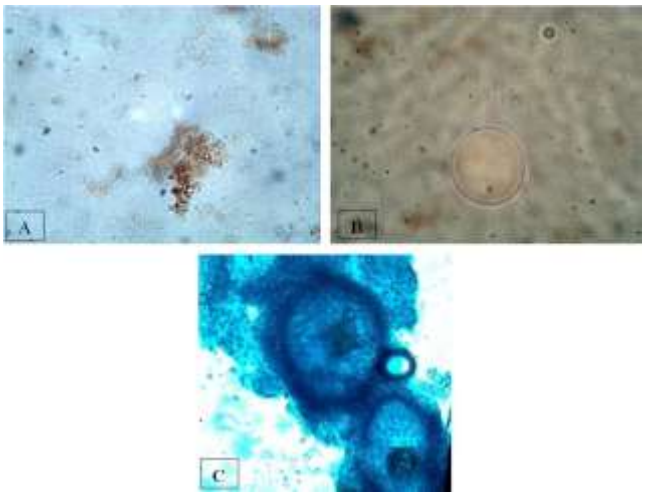

Figure 4. Result of apoptosis examination using immunocytochemistry method. A. Granulosa cell that experiences apoptosis shown by expression of brown color B. Oocyte that experiences apoptosis. C. Cumulus oocyte complex that does not experience apoptosis.

Apoptosis on granulosa cell and cumulus oocyte complex of aceh cattle after in vitro maturation was expressed by brown color on both structures that indicated bond of antigen and antibody visualized by chromogenic.

Discussion

Number of oocytes which was successfully collected was relatively low. It was caused by ovary samples taken were waste from slaughterhouse. Regulation that stated that productive female cattle was forbidden to be slaughtered caused relatively low number of ovary waste. Besides, reproductive status of cattle whose ovary was taken was not surely known. There was possibility that ovary was in luteal phase where follicle growth was not as much as active ovary in follicular phase.

Maturity rate of aceh cattle oocytes which reached only 65.71% was also influenced by another factor such as unfresh ovary due to far distance of slaughterhouse and laboratory where oocyte collection was conducted. In addition, Gordon (1994) stated that a factor which influenced the success of oocyte maturity was how

to take or collect oocyte from ovary. Technique of taking oocyte influenced oocyte quality[5]. Proper technique of taking oocyte will not damage cumulus and oocyte. Besides, culture medium used in the study is also able to influence. The study used DMEM as culture medium. It stated that medium type for oocyte and embryo culture highly influenced every stage of oocyte and embryo growth[6]. In sheep, combined TCM-199 medium was good for oocyte maturation and CR1aa was good for fertilization medium and embryo culture[6]. In some studies, there are several types of medium culture that are able to be used for in vitro maturation besides DMEM, such as KSOM, *T*issue culture medium (TCM)-199, dan *C*harles Rosenkrans (CR)1aa TCM-199 medium used for in vitro embryo production of cattle[7] and culture cattle embryo on CR1aa medium[8,9].

In addition to maturation data, examination on apoptosis that took place in granulosa cell and cumulus oocyte complex of aceh cattle after in vitro maturation was also conducted. Result of examination on apoptosis is presented at Table 2, Figure 3, and Figure 4. Based on the result of the study, out of 30 cumulus oocyte complexes after in vitro maturation, only 13.33% of them experienced apoptosis whereas 86.67% of them did not experience apoptosis.

Brown color showed granulosa cell and cumulus oocyte complex cell that experienced apoptosis so that it would influence nutritional supply from cumulus to oocyte. It led to oocyte disability to have perfect maturity. Whereas, aceh cattle cumulus oocyte complex that did not experience apoptosis was expressed by staining counter color that is *m*ethil green. Cumulus oocyte complex that did not experience apoptosis caused oocyte ability to reach perfect maturity that is metaphase II stage.

Many factors influenced apoptosis to take place and accelerate the process. One of them was medium type used for culture. Medium type was able to influence apoptosis on cumulus cells and was able to reduce oocyte ability to develop to next stage[10]. Based on kinetic research on oocyte maturation it was known that apoptosis was getting higher as oocyte reached metaphase II which[11] likely was caused by *r*eactive oxygen species (ROS)which was increasing too on cumulus cells[12] and oocyte[13]. High *R*eactive oxygen species made cumulus cells and oocyte become toxic and finally experienced death (apoptosis).

Conclusions

Based on the result of the study, it is concluded that aceh cattle oocyte has ability for in vitro maturation in DMEM medium with low apoptosis rate. Aceh cattle oocyte from slaughterhouse waste has potency to produce mature oocyte by applying in vitro maturation technology.

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Declaration of Interest

The authors report no conflict of interest.

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