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Supplement of Acrosin To The Post Thawing Sperm of Sheep Towards Increasing Potency of Spermatozoa

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Abstract

The development of cattle population in Indonesia has not reached in happy condition, even in east Java in the 2001 was decreasing population of some cattle, such as sheep 3,24 %, cow 5,86% and buffalo 5% whereas other cattle have increased far from our hope. (Anonymous, 2007). The obstacle which is faced in the cattle breeding field is involving reproduction field, the priority fertilization problem includes failure sperm cell to penetrate zone pellucid on egg cell due to less enzyme acrosin potency and this is the first factor which hampers class cattle reproduction.

Focus on the problem mentioned, so the short aim of this research is to get specific identify protein from acrosin that is in the acrosom sheep spermatozoa and to isolate specific protein from acrosin as the bioactive material to make better cattle fertility. As for the further aim of this research is to get supplying specific protein from acrosin as the commercial way as well as to make better cattle fertility by making better its class reproduction. This research is laboratorial experiment, it is done by biological potential test from acrosin with spermatozoa medium without acrosin and spermatozoa + acrosin. The investigation of biological potential test including: motility percent, viability, abnormality, capacity, non-capacity and acrosom reaction. The investigation is divided into 2 incubation times, they are 30 minutes and 60 minutes.

The result are: acrosin supplementation 4,5 µgram can increase motility sheep spermatozoa. Acrosin supplementation 3,0 and 4,5 µgram can increase viability, capacity and spermatozoa sheep acrosom reaction EG.

The conclusion are: acrosin supplementation (addition) with dosage 4,5 µgram can be used to increase biological sheep EG potency, so it can be used as alternative (bioactive) material to make better cattle sheep fertility.

Keywords: *Acrosin, Bioactive Material, Biological Potency, Zona Pellucida, fertility*

1. Introduction

The growth of livestock population in East Java has not reached the satisfying result, even in 2007 some livestock populations were declined in number there are sheep & goat 2,24, cow 1,86 % and bull 3 % while the other livestocks were having increase in number that still far from expectation (Anonimous, 2007). The government programs to improve the sheep & goat population are artificial insemination (AI) & natural breeding (NB).

In 2007 the number of newborn goat and sheep through Artificial Insemination (AI) or Natural Breeding (NB) was still below 1 million (Tjeppy Soedjana, 2008). The common problem in attempt to improve the goat & sheep population is fertilization disturbance that include the failure of the sperm cell to penetrate the egg cell (Hafez, 2002).

The failure of the sperm cell to penetrate the egg cell pellucid zone is because the decrease of enzyme *α serine protease (acrosin)* potential, the rate of the failure reach 20-30 (Zalata, 2004; Hafez, 2002). *Acrosin* is a protease enzyme in sperm acrosomal and it is crucial in fertilization process. Acrosin is produced during acrosome reaction, it is shown by the penetration of spermatozoa into ovum pellucid zone.

Acrosin that is produced by acrosom from spermatozoa have a strong influence in penetration of spermatozoa to pellucid zone (PZ) from the egg cell or ovum. Acrosin activation and *plasminogen* as the proteolytic enzyme in sheep's spermatozoa is crucial to induce acrosom reaction and spermatozoa penetration inside the oosit. Vitamine A also work as the activator from acrosin activity and plasminogen.

The acrosom reaction from each areas in epididymis after capacitation is aimed for ovum penetration in sheep (Zervos *et al.* 2005). The factors that are related acrosin activity in spermatozoa can be used to evaluate a male animal's fertility. The capacitation process causing the acrosom reaction to happen, it is an extraordinary change of structure in anterior part of the spermatozoa head that is needed in fertilization of mammal animals (including sheep). This allow the acrosin enzyme release from organel like sac to help in penetration especially in pellucid zone from the egg cell.

Based from the problem, therefore the short-term aim for this research is to test the biologic potential of specific protein (acrosin) in sheep EG spermatozoa acrosome and supplementation from acrosin as a bioactive ingredient to improve the livestock fertility in in-vitro fertilization. The long-term aim for this research is the provision of specific protein from acrosin to improve the livestock fertility for embryo transfer.

2. Materials and Methods

In supplementation research of sheep EG acrosin to biologic potential of centrifuged spermatozoa. In-vitro fertilization was conducted with spermatozoa that had been suplemented with acrosin or without acrosin and the success rate of the fertilization was observed.

2.1 Oosit Aspiration and Observation

Oosit Aspiration Procedure

Before doing the oosit aspiration, the ground and table for the work had to be disinfected with alcohol 70%. The ovarium was washed for 2-3 times in 30^oC degrees of physiologic salt solution, then the ovarium was put on a baker glass and kept in the 38^oC waterbath (Djuwita, dkk. 2005). Next the spuit (inject tool) was filled with 1-1,5 ml Oosit Washing Solution (OWS) media, then the 18G needle was used to inject the ovarium parenkim part near the follicle. The aspiration was done in follicle with 2-5 mm diameter that was near the needle inject point (without taking the needle out), after the follicle liqued was absorbed or the spuit was filled around 3-4 ml, the liquid was moved inside a sterilized reaction tube and kept in the waterbath. The liquid absorption or the movement into the petri dish had to be done with extra care to avoid damaging the cumulus. In every taking, the average oosit that can be stored is 4-5 oosit, meanwhile in every in-vitro fertilization the oosit that was needed were 60-80 ovums.

Observation and Oosit Clasification

The aspiration liquid along with the content inside was poured from reaction tube to petri dish with diameter 90 mm. The petri dish with diameter 35 mm was prepared and filled with OWS media to wash the oosit, then the oosit was pasteur pipette that has slightly bigger diameter than oosit diamter (becareful not to break the cumulus cell). Oosit was put inside the petri dish filled with OWS media and washed for 2-3 times, then the oosit was observed under the microscope to be classified. Only the ossit with cumulus would be implanted in TCM_{199}.

2.1 Ripening In-Vitro Oosit

Every oosit must be washed for 2-3 times in petri dish filled with TCM 199 around 2,5-3 ml. Then the oosits were moved to maturation medium TCM 199 added with 10% serum. At first 80 oosits were put into droplet medium 500 µl, then medium was filled with droplet 100 µl each contained 20-25 oosit and incubated inside the incubator 5% CO2 humidity 95-100%, temperature 38oC for 20-24 hours.

2.2 Oosit Washing

The oosits that had been incubated for 20-24 hours were washed with OWS around 20 minutes before the fertilization. Each group (20-25 oosit) was washed for 2-3 times before counting the mature oosit.

2.3 In-Vitro Fertilization on TCM_{199} Media

The process is similar to insemination, however it was the oosit cell that was inserted. First, drop the spermatozoa that had capacitated for an hour on the TCM_{199} media taken out from the CO_2 incubator. Around 20-25 oosits were put inside a drop of spermatozoa that had been suplemented wih acrosin 0; 3.0; 4.5 and 6.0 µg then incubated with CO_2 incubator for 24 hours in temperature $38,5^oC$.

2.4 Washing the Fertilized Oosit on TCM_{199} Media

The oosits were taken out of the petri dish from the CO_2 incubator then washed by using OWS media 2 times. After that the oosits were moved to a 35 mm diameter petri dish filled with TCM_{199} media

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according to acrosin dose 0; 3.0; 4.5; 6.0 µg. Then counting the success rate of the fertility.

3. Result and Discussion

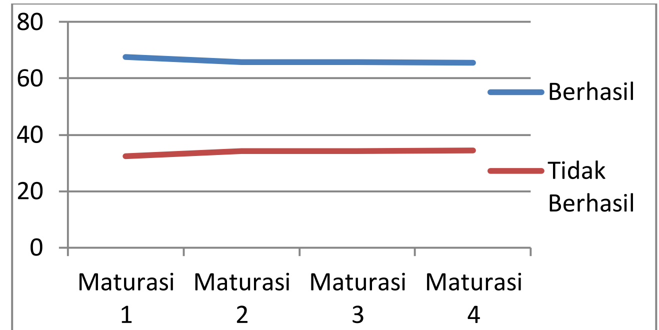
3.1 The Maturation Result of The Sheep Oosit After Incubated for 24 Hours

After 2 times aspiration on sheep follicle ovarium from 78 ovariums, 76 oosits were ready for maturation in CO_2 incubator for 24 hours. The result of the maturation can be seen from the table 4.1 below

Tabel 3.1. Average maturation sheep oosit success percentage

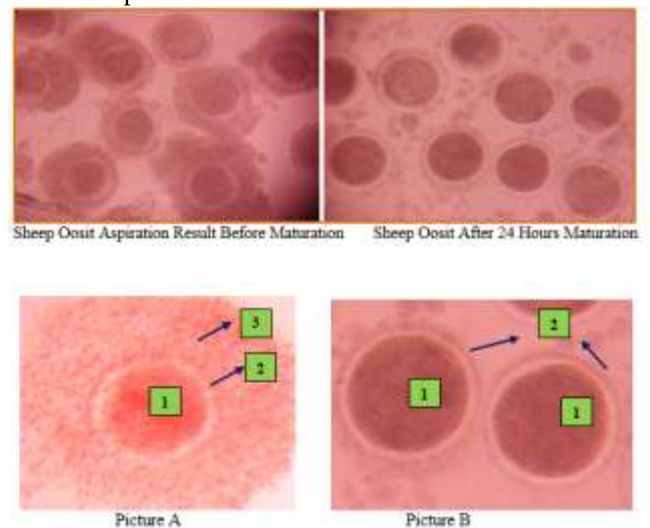
Maturation	Total Maturated Oosit	Maturation Result	Maturation Percentage	X^2
Maturation 1	40	27	67,50	X^2 : 0.099 B : 0.922
Maturation 2	38	25	65,79	
Maturation 3	35	23	65,71	
Maturation 4	32	21	65,62	
Total	**145**	**96**	**Average 66,16**	

From table 4.1. above the average percentage for the success maturaion in the first day and the second day is 66.16%. The number slightly lower than Yanagimachi (1994) did on maturating the cow oosit 75.60%. In general, the maturation success is depend on the environment, maturation media, and the tools (Supriatna dan Pasaribu 2002). The most crucial variable in selecting the tools are : usability, repeatable, and well functioned. Maturation media depend from the composition and the production process, meanwhile the environment that was meant above is the condition where the ovarium was earned in animal slaughterhouse and how long the sheep was slaughtered.



Picture 3.1. Average Percentage of the Sheep EG Oosit Maturation Success Maturation 1, 2, 3 and 4 on TCM $_{199}$ media for 24 hours

After the Chi-Square test was done, the result is there is no conceret difference (p≥0.05) between the maturation result percentage of maturation 1, 2, 3 and 4 (attachment 8). The data above shows that in every sheep oosit maturation, the result will be the same, for more details the result of the maturation can be seen in picture 3.2 below :



Picture 3.2.Oosit before and after maturation in TCM_{199} media maturation for 24 hours
Description :

Picture A : Ovum with cumulus (before maturation)
Picture B : Ovum without cumulus (maturation result)
Number 1 : Core material
Number 2 : Pellucid Zone
Number 3 : Cumulus Oophorus

3.2 Success percentage of cell cleavage in in-vitro fertilization after being suplemented by acrosin

After the oosit had been fertilized for 24 hours in each of acrosin dose 0; 3.0; 4.5 and 6.0 the

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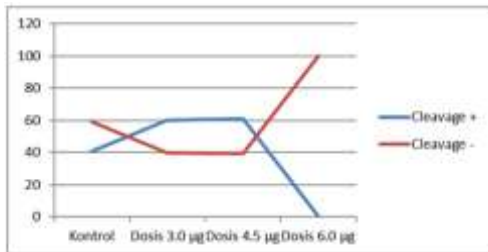
cell cleavage percentage that occurred can be seen in table 4.2 below :

Treatment	Total Oosit After Being Fertilized In-Vitro	In-Vitro Fertilization Result	In-Vitro Fertilization Percentage	X^2
Control	27	11	40,74	X^2 : 21.429
Treatment 3,0 µg	25	15	60,00	P : 0.000
Treatment 4,5 µg	23	14	60,87	
Treatment 6,0 µg	21	0	0	
Total	96	40		

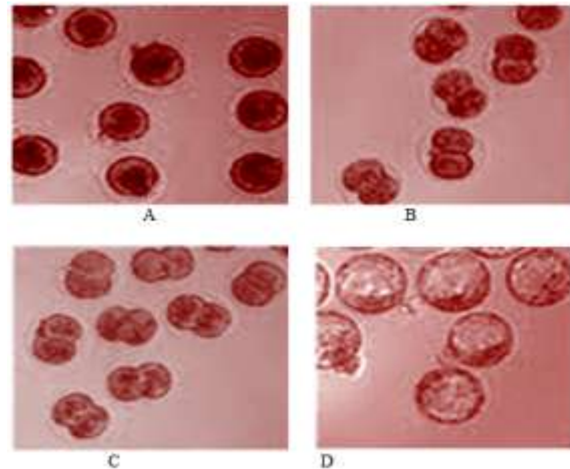
Table 3.2. Average percentage of cell cleavage result in-vitro fertilization after acrosin supplementation

From the table above, the percentage of cell cleavage in acrosin dose 3,0 and 4.5 µg are the biggest compared to other dose, the result shows that acrosin dose 4.5 µg is the optimum dose, meanwhile 3,0 µg dose is enough to generate cell cleavage. In 6.0 µg dose the cleavage does not occur, and the lysis happened in almost of all pellucid zone. The data shows that acrosin is crucial and needed to generate the proteolysis in egg cell pellucid zone (Adel, *et al.* 2004).



Picture 3.3.Average Percentage cell cleavage on sheep EG oosit after in-vitro fertilization for 24 hours

The result of Chi-Square test shows that there is a concrete difference ($p \leq 0.01$) pada persentase cleavage antara dosis acrosin 0; 3.0; 4.5 dan 6.0 µg. This shows that acrosin supplementation in dose 3.0 and 4.5 capable to improve the in-vitro fertilization rate, while in dose 6.0 the cleavage did not occur, more details can be seen in picture 4.4 below :



Picture 3.4. Fertilization In-Vitro Result in Sheep EG Oosit with Acrosin Supplementation

Description

A. Fertilization In-Vitro result without acrosin supplementation
B. Fertilization In-Vitro result with acrosin supplementation 3.0 µg
C. Fertilization In-Vitro result with acrosin supplementation 4.5 µg
D. Fertilization In-Vitro result with acrosin supplementation 6.0 µg

From the data above the quadratic relation can be seen on acrosin adding for cell cleavage. Improving at first, however the bigger the dose the result declined. Acrosin adding is needed to improve the potential of penetrating the oosit pellucid zone, thereby the in-vitro fertilization rate become better (Adel, *et al,* 2004; Primakoff & Myles, 2004). Acrosin dose 6.0 µg causing lysis in almost of all pellucid zone and resulting in the failure of fertilization. The previous researches showed that the number of acrosin has positive correlation with the number of fertilization, low acrosine dose in spermatozoa resulting the failure of penetrating the pellucid zone (Adel, *et al*. 2004; Hafez, 2002).

4. Conclusion

4.1 Summary

Based on the result and the assesment from this result, the conclusion is :

- Acrosin supplementation dose 3.0 µg with 30 minutes incubation on sheep EG cement will improve the spermatozoa potential for the cleavage to happen in in-vitro fertilization.

4.2 Suggestion

- Acrosin suplementation dose 3.0 and 4.5 µg with 30 minutes incubation can be used to improve the sheep EG spermatozoa biology potential, thereby the spermatozoa can be used for sheep in-vitro fertilization.

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