ACROSIN HALF-BREED ETAWA GOAT (PE) SPERM CHARACTERISTIC TO INCREASE SPERMATOZOA QUALITY

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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 goat 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 goat 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 goat PE's spermatozoa. Acrosin supplementation 3,0 and 4,5 μgram can increase viability, capacity and spermatozoa goat acrosom reaction PE. The conclusion are: acrosin supplementation (addition) with dosage 4,5 μgram can be used to increase biological goat PE potency, so it can be used as alternative (bioactive) material to make better cattle goat fertility.
Key words: Acrosin, Bioactive Material, and Biological Potency.

INTRODUCTION

Acrosin is protease enzyme found on akrosomal sperm and has vital importance for fertilization process. Acrosin is spent during acrosome reaction, this shown with attachment of spermatozoa to zone pellucid ovum or penetration of spermatozoa into pellucid zone. Low rate from acrosin will cause infertility and subfertilitity at livestock. From research which have former done indicate that the amount of acrosin has positive correlations with the numbers of occurence of in-vitro fertilizations. Low rate acrosin at spermatozoa cause failure of penetration at egg cell (Adel A. Zalata, et al. 2004). Activity from acrosin at spermatozoa from a semen visible based on concentration of sperm, motility percentage and also sperm morphology and character.

This enzyme (acrosin) is trypsin-like serine proteinase which at spermatozoa acrosome. It is an important proteolytic enzyme available for pellucid zone hydrolysis from ovum and very vital in the process of fertilization and also have an effect on to acrosome reaction. Acrosin formed by serine proteinase from spermatozoa acrosome and involved in is acrosome reaction in spermatozoa capacities for penetrating into pellucid zone (Williams, RM, JK Graham and RHODIUM Hammerstedt. 2001). Many evidences that acrosin are important for fertilizations process (Goodpasture JC; KL.Polakoski; JD. Zaneveld. 2000).

At frozen semen (sperm) for marrying injection (artificial insemination) at cow or goat, where too old storage and less controlled temperature, will influence the quality of frozen sperm. This thing is because

activity from acrosin enzyme will become decreased. On that account in this research will be done measurement of acrosin rate at frozen sperm with seeing the biological potency, cover sperm motility test, viability, fully plasma membrane, fully acrosome cap, capacitation, acrosome reaction, and also penetrability to zone pellucid (ZP).

MATERIAL AND METHOD

This research is to know the role of acrosin at halfblooded etawa goat spermatozoa to motility percenttage, viability, membrane integrity, capacitation, and spermatozoa acrosome reaction. Material which applied is acrosin isolate from halfblooded etawa goat spermatozoa elusion result from research of phase I, semen goat which accomodated from halfblooded etawa goat (counted 10 tail) in healthy condition and have high libido, condensation hipoosmotik 0,032 M (7,35 g Sodium citrate of 2H20, 13,52 g fructose which dissolved into 1 litre aquades), formalin, eosin negrosin, NaCl 3%, NaCl is physiological, coloration of Chlortetracyclin (CTC CTC, Fixative, DABCO, trichloroacetic acid of 20%, HCl 1N, Sodium thiobarbiturat 1%).

Appliance which applied is scale tube, sentrifuge tube, sentrifuge tools, object glass, [cover/conclusion] glass, microscope, incubator, contrast phase microscope, fluoresencece microscope, spectrophotometer. Semen from male goat accomodated by using made vagina in which provided with is scale receiver glass tube. Made vagina were prepared with installing both receiver appliance and shroud which have been sterilized, while room between external and internal shrouds filled with water which heated until temperatures of 45°C with a purpose to giving temperature to shrouds in equal to 42-43°C and one-third frontages camouflaging in vaginas made is nubed with vaselin. Here in after male goat given by excitement by female of angler then done relocation of semen. Soon after relocation, semen brought to laboratory for checked.

Inspection of fresh semen quality and inspection of semen after disuplementation with acrosin

Inspection of fresh semen quality was done as soon as possible after relocation. Inspection of fresh semen quality consisted of inspection of macroscopic and microscopic, that is : volume, colour, consistency, hydrogen ion exponent, motility, viability, abnormalitity, capacitation status and membrane integrity. Inspection of spermatozoa after disuplementation with acrosin including : motility, viability, abnormalitity, capacitation status and membrane integrity.

Inspection of makroskopis :

Volume : Volume of semen directly knowable from semen which seen at scale receiver tube. **Colour :** Visible semen colour directly momentary after relocation. **Consistency** : Consistency of viscosity checked by shaking down containing tube semening inch by inch and at the same time see surface mobility semening in tube. If friction of semen in the movement tube was interpreted tardy as semen having viscid consistency, while if the movement was quickly interpreted semen having watery consistency. **Degree of acidity (hydrogen ion exponent)** : Measurement of semen hydrogen ion exponent done by dripping semen by using ose at indicator hydrogen ion exponent paper. Discoloration at indicator hydrogen ion exponent paper reconciled with hydrogen ion exponents standards colours.

Microscopic Inspection cover :

Individual motility : Indivudual motility of spermatozoa is observed soon after done relocation of semen. Observation done by using magnifycation light microscope of 10 x 40 expanded at as endue thin semen above object glass. **Mass motility :** Mass motility is observed less than 10 minute after semen accomodated with light microscope of 10 x 10 expanded.

Concentration : Examination of semen hypothesis used spectrophotometer. The process is as follows : NaCl 3% taken with micropipette or scorex counted 3,98 ml and packed into cuvet tube which have been filled by 0,02 ml semen then homoginated with thermomixer.

Spermatozoa abnormalitity and viability : Viability observation (% of alive spermatozoa) and spermatozoa abnormalitity done by using coloration of differentiation that is making comment preparat which coloured with eosin-negrosin.

Integrity membrane spermatozoa by using condensation HOS test : Spermatozoa membrane integrity tested with condensation hypoosmotic swelling test (HOS tested). Counted 100µ semen levator skapula mixed with 1 ml condensation hipoosmotik (0,49 g citrate sodium x 2H2O and 0,9 fructose in 100 ml aquades). Those condensation incubated at temperature 37°C for 30 minutes. Spermatozoa membrane observed with light microscope of 400 times expanded ; to the distension of spermatozoa and existence of rolled tail (Fonseca, et al., 2005; Light, et al., 2005).

Capacitasy Spermatozoa Status : Examination of capacitation status (capacitation, acrosome reaction and non capacitation), good fresh semen and also semen treatment done with coloration of Chlortetracycline (CTC Staining). Observation with epifluourescence microscope (Nikon Microscope OPTIPHOT-2 apply filter-UV2A consisting of excitation filter EX330-3, dichonic mirror DM400 and Barrier filter BA435) using ultraviolet light source (Sumitro and ethic, 1998).

Acrosome Status at Spermatozoa : Spermatozoa fixation by 4% formaldehyde, then cleaned by adding PBS 3 ml and sentrifuged 1500 rpm for 10 minutes, supernatant thrown and added by 0,3 ml FITC con A (Sigma) with concentration of 10µ g/ml in PBS dulbeccos. Observation shown: (a) spermatozoa with acrosome intak, and (b) spermatozoa without acrosome. This method is researcher modification result from method before all (Ethic, 2000) from Nishikima method (1997).

RESULT AND DISCUSSION

Semen taken as research sample for acrosin disuplementation have criteria as competent semen to be weared as artificial insemination at goat, that is white milk chromatic and a little bit of cream brass colour, hydrogen ion exponent of 6-7, mass motility of 2+ - 3+, spermatozoa individual motility ≥ 70% (Zenichiro, et al., 2002). Out of 10 PE male goats which the semen was taken by made vagina in the result is as described at Tables of 1.

Tables of 1. indicate that mean of fresh volume percentage from this research result is 1,12 ± 0,25 ml, much the same to with obtained by Garner and Hafez (2000) that volume of goat semen in tropical area. Ranging from 0,8 - 1,2 ml. Jainudeen, et al., (2000) express that semen volume and amount of spermatozoa which yielded from every ejaculations influenced by specieses, age, season, area, ejaculation frequency, condition of health and food.

Goat semen colour generally white milk chromatic and a little bit krem brass colour. Various of this brass colour relate to existence of contents of riboflafin which yielded from gland secretion vesicula seminalis (Lindsay, et al., 1982; Evans and Maxwell, 1987).

Research result of spermatozoa consistency generally condensed. visually progressively condensed semen shown concentration of spermatozoa in excellent semen (Mcdonald and Pineda, 1989).

Table 1. Characteristic Fresh Semen Half-Breed Etawa Goat (PE)

Parameter	Mean ± SD
Colour	Milk white
Consistensi	Solut
Ph	6,56 ± 0,25
Volum (ml)	1,12 ± 0,25
Concentration (10^6)	2326,91 ± 106,14
Motility Mass	$2^+ - 3^+$
Motility Indiv (%)	75,56 ± 2,96
Viability (%)	92,50 ± 3,67
Abnormality (%)	5,56 ± 2,40
Integrity Membrane (%)	65,89 ± 7,22
Status Capasitation:	
- Status Capasitation (%)	57,55 ± 4,22
- Capasitation (%)	24,42 ± 8,97
- Reaction of Acrosome (%)	18,34 ± 5,75

Concentration of spermatozoa which obtained from this research is 2326,91 ± 106,14 x 106 spz/ml. Mean percentage concentration of this pertained is normal. Garner and Hafez (2000) express that concentration of goat spermatozoza ranging from 2.000 - 3.000 x 106 spz/ml. Devendra and Burns (1994) express that concentration of fresh semen goat per ejaculate ranging from 1,8 - 4 x 10 106 spz/ml. Degree of acidity (hydrogen ion exponent) share to determine status of life of spermatozoa in semen. Mean of fresh semen goat hydrogen ion exponent which obtained from this research is 6,56 ± 0,14. The pH value stay a few under normal hydrogen ion exponents, as said by Garner and Hafez (2000) that goat have hydrogen ion exponent ranging from 5,9 - 7,3.

The mean PE goat spermatozoa motility percentage from this research is 75,56 ± 2,96 %. This show is higher compared to the spermatozoa motility reported by Tambing, dkk. (2000) that goat spermatozoa motility FE is 74,29 ± 2,70%%,, however a few lower than which reported by Wicaksono (1999) that goat spermatozoa motility is 87,88± 2,61 %. Motility is valuation of quality of which in character subjectif because based on decision of examiner individual (Bearden and Fuquay, 1997). The good spermatozoa motility is seen from mass motion form thick cloud and the moving is progressive of active individual forwards (Ethic, 2007).

The mean of goat spermatozoa membrane integrity percentage from this research is 65,89 ± 7,22 %. This result is compared lower which reported by Isnaini (2006) that PE goat membrane integrity is 72,33 ± 3,66 %. Degradation of quality of spermatozoa can happened because biological factor and also because factor influence prosesing semen.

Result of research of capacitation, acrosome reaction and non fresh semen spermatozoa capacitation each is 23.42 ± 8,97%%,, 18,34 ± 5,75 % and 57,55± 4,22 %. This result are a bit better from which reported by Zamanti (2003) that capasitation is 24,25 ± 0,10%%,, acrosome reaction that is 11,83 ± 0,14 % and non capacitation that is 64,23 ± 0,21. Contents of fructose in goat semen that is 250 mg/100 ml, lower than cow semen that is 460 - 600 mg/100 ml (Garner and Hafez, 2000). Yanagimachi (1994) express that forest pig and goat only require time of 1 until 2 hour(clock for reaching optimal spermatozoa capacitation).

Tables of 2. Influence Suplementation Acrosin and Long of Incubation to Motility of PE Goat Spermatozoa

Doses acrosin (µ gr)	Long Incubation (minute)	Mean Motility of Goat Spermatozoa (%)
0	30	67,10 ± 5,29[a]
	60	65,34 ± 7,65[a]
3,0	30	67,81 ± 4,19[a]
	60	68,18 ± 4,81[a]
4,5	30	68,90 ± 8,59[b]
	60	70,20 ± 6,34[b]
6,0	30	53,50 ± 9,81[c]
	60	55,12 ± 6,26[c]

description : Different notation at same column, showing to differ in reality (p)

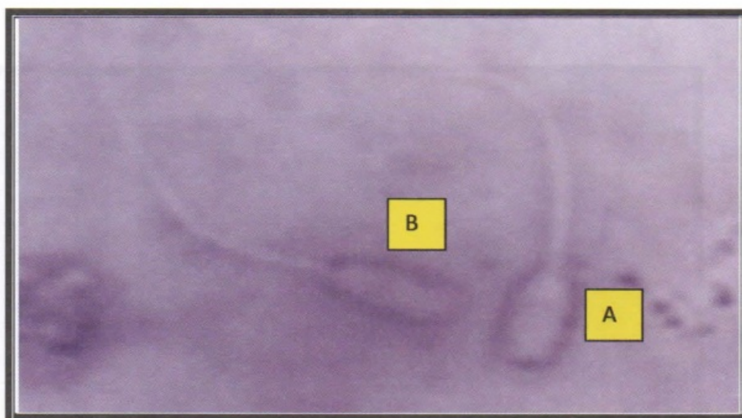
Characteristic of difference of influence acrosin suplementation and a long time of incubation continued with Reality distance test Duncan (JND) result indicate that treatment without acrosin and treatment with acrosin 3,0µ gram at various level incubations strippers shown to have an effect on not be real ($p>0,05$) to mean of goat spermatozoa motility percentage PE to mean of goat spermatozoa motility percentage analysis . While treatment with concentration of acrosin 4,5 and 6,0µ gram at various incubations shown to have an effect on reality (p) to mean of PE goat spermatozoa motility percentage. Biologically that treatment of acrosin 4,5µ gram resulting higher mean of goat spermatozoa motility percentage from other treatment. This is because addition of acrosin can reduce forming of lactic acid in cell causing at increasing of hydrogen ion exponent, causing metabolic process become to increase, and cause increasing of forming of ATP, so that will increase spermatozoa motility (Jones, et al., 1992; Garner and Hafez, 2000; Rigau, et al., 2002; Ford, 2006).

Spermatozoa living have membrane which intak (Draw 3), cause eosin dye negrosin have no admission pass membrane. Membrane function to protect organ in cell and as filter at surface of intraseluler and ekstraseluler. Died spermatozoa (draw 4.2B) show the plasma membrane destroyed. This situation cause cell metabolism process annoyed and membrane permeability become very high so that eosin dye negrosin can pass membrane and cause on fatal for spermatozoa (Saili, 1999).

Tables of 3. Influence Suplementation Acrosin and Long of incubation to Viability of PE Goat Spermatozoa

Dosis Acrosin (µ gr)	Long Incubation (minute)	Mean Viability of Goat Spermatozoa (%)
0	30	82,19 ± 4,64[a]
	60	78,60 ± 3,45[a]
3,0	30	87,20 ± 2,79[b]
	60	85,48 ± 4,76[b]
4,5	30	85,23 ± 3,89[b]
	60	83,20 ± 6,34[b]
6,0	30	76,50 ± 9,81[c]
	60	74,12 ± 6,26[c]

description : Different notation at same column, showing to differ in reality (p)



Draw 1. Result observation of goat spermatozoa viability PE by light microscope, description : A. spermatozoa lived (transparent) and B. dead spermatozoa (purple)

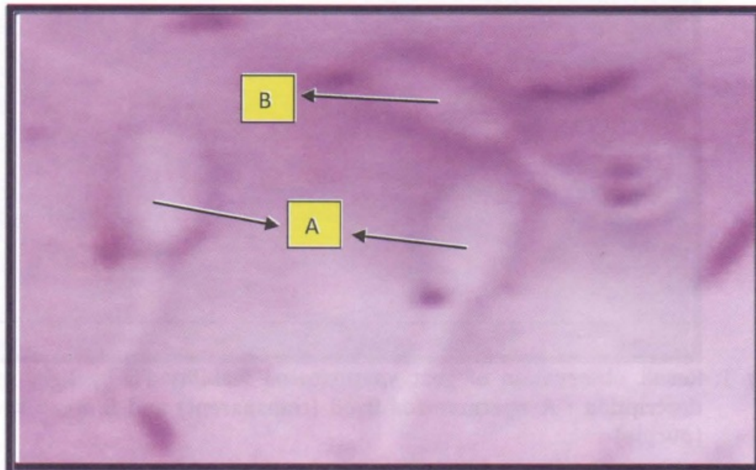
Analysis result indicate that treatment without acrosin suplementation and treatment with acrosin suplementation 3,0; 4,5 and 6,0µ gram at incubation have an effect on not be real ($p>0,05$) to mean of PE goat spermatozoa viability percentage. Biologically indicate that treatment of acrosin suplementation 3,0µ gram at various incubations resulting mean of PE goats spermatozoa's viabilities percentages better from other treatment. This indicating that acrosin suplementation up to 4,5µ gram in goat semen PE still give better effect to life of spermatozoa. acrosin suplementation 6,0µ gram have shown degradation of mean PE goat spermatozoa viability percentage. This is anticipated to relating of forming of lactic acid (Hafez, 2000).

During incubation, spermatozoa experienced changed of membrane compiler molecule causing membrane destabilisation which can reduce physiological function of membrane. Bearden and Fuquay (1997) express that activity metabolic semen became maximum if diluted with isotonic diluent, however if diluent had the character of hypertonic or hypotonic will reduce speed of metabolism but will not increase the life spermatozoa.

Tables of 4. Influence Acrosin Suplementation and long of incubation to Abnormality of PE Goat Spermatozoa

Doses acrosin (µ gr)	Long Incubation (minute)	Mean Abnormality of Goat Spermatozoa (%)
0	30	$7,40 \pm 3,76^{a}$
	60	$8,87 \pm 2,49^{a}$
3,0	30	$6,44 \pm 2,19^{a}$
	60	$7,38 \pm 2,09^{a}$
4,5	30	$8,23 \pm 3,30^{a}$
	60	$9,44 \pm 2,36^{b}$
6,0	30	$8,87 \pm 2,45^{a}$
	60	$9,96 \pm 1,67^{b}$

description : Different notation at same column, showing to differ in reality (p)



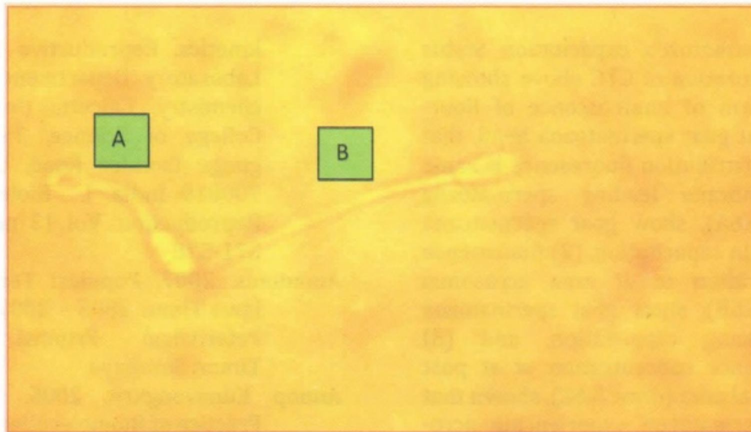
Draw 2. Result observation of PE goat spermatozoa abnormalitity by light microscope phase contras, description : A. Spermatozoa fully (all part of perfect spermatozoa body and chromatic transparent) and B. abnormal spermatozoa (part of body is not normal with imperfect tail formation

Result observation of PE goat spermatozoa abnormalitity with coloration of eosin negrosin observable at microscope phase contras of 1000 times expanded at picture of 2. Analysis result indicate that treatment without acrosin suplementation and treatment with acrosin suplementation dose of 6,0μ gram at various incubations have an effect on not be real (p>0,05) to mean of PE goat spermatozoa abnor-malitity percentage, while acrosin su-plementation dose of 3,0 and 4,5μ gram at various incubations strippers have an effect on reality (p) to mean of PE goat spermatozoa abnormalitity per-centage. The mean of PE goat abnor-malitity percentage which obtained from this research less than 10. This indicate that process of handling of semen from relocation until done by suplementation technically have been put accross, so that secondary abnor-malitity can be depressed as low as possibly.

Tables of 5. Influence of Acrosin Suplementanio and long of incubation to Integrity of PE goat Spermatozoa Membrane

Doses Acrosin (μ gr)	Long Incubation (minute)	Mean of Integrity Membrane of Goat Spermatozoa (%)
0	30	46,40 ± 5,76[a]
	60	45,87 ± 4,49[a]
3,0	30	46,44 ± 4,19[a]
	60	46,38 ± 5,09[a]
4,5	30	45,23 ± 3,30[a]
	60	45,44 ± 2,36[a]
6,0	30	45,87 ± 2,45[a]
	60	44,96 ± 1,67[a]

description : Different notation at same column, showing to differ in reality (p)

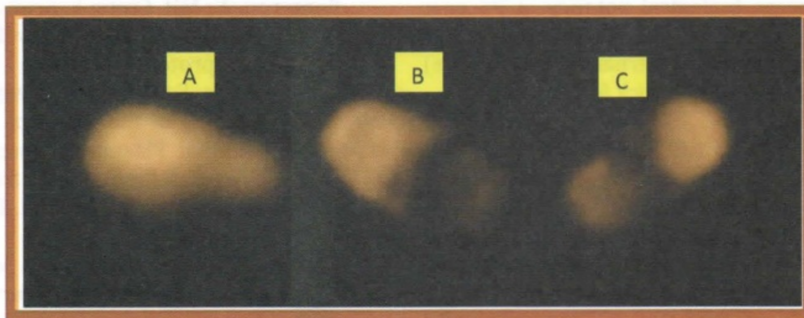


Draw 3. Result Observation of Goat Spermatozoa Membrane Integrity by Light Microscope Phase Contras, discription : A. spermatozoa with intake membrane (circle tail), and B. spermatozoa with demage membrane (tail straight). 400x

Tables of 6. Mean of PE goat Spermatozoa Capasitation Status Percentage after Acrosin Suplementation and long of incubation.

Perlakuan		Capasitation (%)	Reaction of Acrosom (%)	Non Capasitation (%)	Total (%)
Acrosin (µgram)	Incubation (minute)				
0	30	53,45±9,91[a]	7,66±3,40[a]	38,58±9,23[a]	99,69
	60	61,64±10,46[a]	5,61±2,08[b]	32,75±9,19[a]	99,90
3,0	30	61,91±11,60[a]	9,17±4,55[c]	27,71±4,08[b]	98,79
	60	70,95 ±11,11[b]	8,45±4,21[c]	20,59±7,81[b]	99,99
4,5	30	76,58±17,77[b]	8,98±4,02[c]	10,28±9,09[c]	95,84
	60	80,92±9,39[c]	11,56±4,03[d]	7,52±7,65[c]	100,00
6,0	30	53,71±15,56[a]	4,40±1,02[b]	41,87±5,05[a]	99,98
	60	55,68±18,59[a]	5,52±1,64[b]	36,34±7,62[a]	97,54
Mean					99,24

description : Different notation at same column, showing to differ in reality (p)



Draw 4. Result observation of goat spermatozoa capacitation status at thinning process with coloration of CTC with epiflourescence microscope (Bar =½ µ½ m), description : A. non kapasitasi, B. and capacitation C. acrosome reaction.

goat spermatozoa capacitation Status from coloration of CTC above showing three form of luminescence of flouresence at goat spermatozoa head, that is (1) distribution fluoresence is same at membrane leading spermatozoa (draw 4.6A), show goat spermatozoa which non capacitation, (2) flouresence concentration of at area acrosomal (draw 4.6B), show goat spermatozoa experiencing capacitation, and (3) flourescence concentration of at post acrosomal area (draw 5.6C), shown that goat spermatozoa experiencing acrosome reaction. Result of luminescence of flouresence at goat spermatozoa (draw 4.6.) is image of distribution Ca^{2+} at spermatozoa head membrane, like have been reported by Mattiollli, et al (1996), Caul et al (1997) and ethic (2005).

CONCLUSION

Based on solution and result from this research is inferential as follows : Suplementation acrosin 4,5μ gram can increase PE goat spermatozoa motility, Suplementation acrosin 6,0μ gram can reduce PE goat spermatozoa motility. Suplementation acrosin 3,0 and 4,5 μ gram can increase viability, PE goat spermatozoa acrosome reaction and capacitation. Suplementation acrosin 3,0 and 4,5μ gram can reduce non PE goat spermatozoa capacitation. Suplementation (addition) acrosin with dose of 4,5μ gram applicable to increase biological potency of PE goat spermatozoa, so that usable as component of alternative (bioaktive) for repair of livestock fertility (goat).

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