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PROCEEDING

International Seminar

THE ROLE OF VETERINARY SCIENCE
TO SUPPORT MILLENNIUM DEVELOPMENT GOALS
and

THE 12th ASIAN ASSOCIATION OF VETERINARY SCHOOLS CONGRESS



FACULTY OF VETERINARY MEDICINE
UNIVERSITAS AIRLANGGA



CERTIFICATE OF ATTENDANCE

This is to certify that

Budi Utomo

AS SPEAKER

in **INTERNATIONAL SEMINAR
THE ROLE OF VETERINARY SCIENCE
TO SUPPORT MILLENNIUM DEVELOPMENT GOALS**

*was held in JW Marriott Hotel Surabaya - Indonesia
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President of AAVS
Dean of Faculty Veterinary Medicine
Universitas Airlangga - Indonesia

Prof. Hj. Romziah Sidik., DVM., Ph.D.



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VETERINARY SCHOOLS CONGRESS
JW MARRIOTT HOTEL, SURABAYA-INDONESIA
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**REMARKS OF ORGANIZING COMMITTEE
THE ROLE OF VETERINARY SCIENCE TO SUPPORT
MILENIUM DEVELOPMENT GOALS**

Dr. Dadik Raharjo, M.Kes, DVM.
Chairman

Ladies and Gentleman,

I have the honour of welcome delegates and speakers in International Seminar with the title "role of veterinary science to support milenium development goals" and highest ours appreciation for Your participation on this seminar.

The seminar will exchange information that we can carefully increasing the role of veterinary science to support development goals. Hopefully through this event will take advantage of the many opportunities to colloborative work between indonesia instituion and also with overseas institution.

On behalf of Organizing committe, I would like to express our sincere gratitude and thanks to all participant at this seminar international.

I hope that this program will be useful and enjoy during stay in Surabaya.

Best Regards



REMARK OF DEAN FACULTY OF VETERINARY MEDICINE UNIVERSITAS AIRLANGGA AAVS PRESIDENT

Prof. Romziah Sidik, Ph.D., DVM.

Bismillahi rochmanir rochim,
Assalamu'alaikum warochmatullahi wabarokatu.

Good morning Ladies and Gentlemen,
Welcome to Surabaya, East Java – Indonesia.

On behalf Faculty of Veterinary Medicine, Universitas Airlangga and Asian Association of Veterinary Schools, I would like to say thank you for the Excellencies: Rector Universitas Airlangga, The Director General of Livestock and Animal Health-Ministry of Agriculture-Republic of Indonesia: Ir. Syukur Iwantoro, MS), The Coordinating Minister for people's Welfare Republic of Indonesia: Dr. Agung Laksono; The OIE Sub Regional Representation for South-East-Asia delegates (Dr. Dirk Van Aken, Dr. Mary Joy Gordoncillo, Dr. Ronello Abila and Ms.Melada Ruengjumroonnath), the Presidents of SEAVSA (Dr. Srihadi agung Priyono) President of IVSA (Indonesian Veterinary School Association): Prof. Made Dhamriyasa, and all Deans of SEAVSA (South-East Asia Veterinary School Association) members, AAVS (Asian Association of Veterinary Schools: Japan, Korea, Taiwan, Indonesia, Malaysia, Thailand, Philippines, Mongolia, Vietnam, Myanmar, Lao and Cambodia) and IVSA (Indonesian Veterinary School Association), The President of Indonesia Veterinary Medicine Association: DVM.Wiwiek Bagja), Quarantine and Inspection Agency Commissioner of Korea: Prof Yong Ho Park), Secretary General and Asian Society of Zoo and Wild Life Medicine: Dr. Kimmura Junpei; All the invite speakers comes from: Faculty of Medicine, Faculty of Veterinary Medicine and Tropical Disease Center of Universitas Airlangga, Feed Technology and Nutrition, Research Institute for Animal Production,-Indonesia, College of Veterinary Medicine Murdoch University, Division of Molecular Medicine and medical Genetic, Department of Pathology, Kobe University, Universiti Putra Malaysia, Graduate School of Agricultural and life Sciences University of Tokyo Japan;

The honorable of all presenter and participants, also the sponsorships who are joint in the International Seminar with the themes: "The Role of Veterinary Science to Support Millennium Development Goals and the 12th Asian Association of Veterinary Schools Congress" during 2 days (5th-6th September 2013), which is Faculty of Veterinary Medicine of Universitas Airlangga as the hosted of the event.

Ladies and Gentlemen,

About 193 United Nation member states and at least 23 international organizations declared Millennium Development Goals (MDGs), and they have agreed to achieve the nine MDGs such as: eradicating extreme poverty and hunger, universal primary education, promoting gender quality, and empowering women, reducing child mortality rates, improving maternal health, combating HIV /AIDS, malaria and other diseases, ensuring environmental sustainability, and developing a global partnership for development.

Animal diseases which form an epizootic (Apthae epizootic, mad cows diseases) and or zoonotic like Avian Flu, SARS (Severe Acute Respiratory Syndrome), Salmonellosis, Brucellosis, tuberculosis, rabies are threat to global security warned by Director General of the Word Organization as well as World Animal Health Organization (OIE). These diseases have potentially disastrous consequences if it's not eliminates at their primary source. As we know that about systemic review of 1,415 pathogens are known about 61% infects humans.

To combat and fighting zoonosis diseases, Indonesia has launching the National Commission of Zoonosis Control under Coordinator Minister for people's Welfare Republic of Indonesia.

So, the Veterinary Medicine Schools in Asian country has responsibility to provide some courses in the curricula to achieve Day one competencies. Four pillars could be strengthening by Veterinary School such as: education system, research, public extension and or services, and collaborations. The quality assurance should be guaranteed by each Veterinary Schools. In the event of AAVS congress programs to produce and launch the Logo of AAVS, and the consequence to be added the logo profile and philosophy in AAVS by Law. The other program is to perform Veterinary school curricula and gap analysis. Therefore, Veterinary school curricula in Asian country could be standardized.

On behalf Organizing Committee, I would like to say thank you to Director Research and Public Community Services Board of Directorate General of Higher Education, Ministry Education and Culture Republic of Indonesia, The OIE SRR SEA, Faculty of Veterinary Medicine Universitas Airlangga, IVSA, and the sponsorships from veterinary industries for supporting finance that the event become perform by successfully.

Ladies and Gentlemen,

Again, I would like to say thank you for your participative to the event, and please follow and enjoy the programs as well as your visit in Surabaya by happiness.

Billahi taufik wal hidayah, Wassalamu'alaikum warohmatullahi wa barokatu.



REMARKS OF RECTOR OF UNIVERSITAS AIRLANGGA
Prof. Dr. H. Fasich, Apt.

Assalamu'alaikum Warahmatullahi Wabarakatuh

First of all, let us pray to Allah SWT that because of His blessings we are able to be here in this very important event.

Secondly, I would like to say to all participants: Welcome to Surabaya, East Java, Indonesia!

It is indeed a great honour for me to have the opportunity to be among the participants of this very special occasion, where all of us are going to have in-depth discussion about a very important and interesting topic closely related to veterinary science and the millennium development goals as a way to increase the quality of human health.

Indonesia's Millennium Development Goals (MDGs) are based on the eight international development goals that were officially established following the Millennium Summit of the United Nations in 2000, one of touches on the effort to combat wide-spread diseases such as HIV/AIDS and diseases transmitted by animals such as malaria, avian flu, swine flu, and so forth, which could be a serious threat to global security and human development.

Therefore, concerns over these MDGs from the point of view of veterinary science, especially among the researchers, have to be raised these day. There are numerous recent for conducting scientific research and other scientific activities to bring the MDGs to a success.

In this very special event, I would like to express my deepest appreciation to all members Asian Association of Veterinary Schools for their success in conducting better and better collaborations. Such collaborations are a pre-requisite for all efforts in improving performances, including the standardization of veterinary curricula in the ASEAN region and among Asian countries, in controlling the spread of zoonosis, and in developing and improving bio safety, bio security, surveillance, animal health and animal production.

I strongly believe and hope that this seminar and congress will be able to strengthen the existing networks that occurred among all the members of the association, as the main step in the eradication and prevention of infectious diseases, especially once that are related to animals, to support the Millennium Development Goals.

To all participants, I would like to thank you very much for coming to this forum. And to the organizing committee, I would like to give my sincerest appreciation for their wonderful job and hardwork in organizing this event.

I hope the seminar and the congress will be fruitful to all of use and lastly, please enjoy your stay in Surabaya.

Thank you very much,

Wassalammu'alaikum warahmatullahi Wabarakatuh.

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

Budi Utomo

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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 subfertility at livestock. From research which have former done indicate that the amount of acrosin has positive correlations with the numbers of occurrence 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 percentage, 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 2H₂O, 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, abnormality, capacitation status and membrane integrity. Inspection of spermatozoa after disuplementation with acrosin including : motility, viability, abnormality, 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 : Individual motility of spermatozoa is observed soon after done relocation of semen. Observation done by using magnification 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 homogenated with thermomixer.

Spermatozoa abnormality and viability : Viability observation (% of alive spermatozoa) and spermatozoa abnormality 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 100 μ 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 epifluorescence 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 10 μ 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 $\geq 70\%$ (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 $1,12 \pm 0,25$ 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 riboflavin 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 $2326,91 \pm 106,14 \times 10^6$ spz/ml. Mean percentage concentration of this pertained is normal. Garner and Hafez (2000) express that concentration of goat spermatozoa ranging from $2.000 - 3.000 \times 10^6$ spz/ml. Devendra and Burns (1994) express that concentration of fresh semen goat per ejaculate ranging from $1,8 - 4 \times 10^6$ 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 $6,56 \pm 0,14$. 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 $75,56 \pm 2,96$ %. This show is higher compared to the spermatozoa motility reported by Tambing, dkk. (2000) that goat spermatozoa motility FE is $74,29 \pm 2,70\%$, however a few lower than which reported by Wicaksono (1999) that goat spermatozoa motility is $87,88 \pm 2,61$ %. 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 $65,89 \pm 7,22$ %. This result is compared lower which reported by Isnaini (2006) that PE goat membrane integrity is $72,33 \pm 3,66$ %. 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 $23,42 \pm 8,97\%$, $18,34 \pm 5,75$ % and $57,55 \pm 4,22$ %. This result are a bit better from which reported by Zamanti (2003) that capacitation is $24,25 \pm 0,10\%$, acrosome reaction that is $11,83 \pm 0,14$ % and non capacitation that is $64,23 \pm 0,21$. 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 Supplementation 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 \pm 5,29 ^a
	60	65,34 \pm 7,65 ^a
3,0	30	67,81 \pm 4,19 ^a
	60	68,18 \pm 4,81 ^a
4,5	30	68,90 \pm 8,59 ^b
	60	70,20 \pm 6,34 ^b
6,0	30	53,50 \pm 9,81 ^c
	60	55,12 \pm 6,26 ^c

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

Characteristic of difference of influence acrosin supplementation and a long time of incubation continued with Reality distance test Duncan (JND) result indicate that treatment without acrosin and treatment with acrosin 3,0 μ 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 6,0 μ 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 4,5 μ gram resulting higher mean of goat spermatozoa motility percentage from other treatment. This is because addition of acrosin can reduce forming of lactic

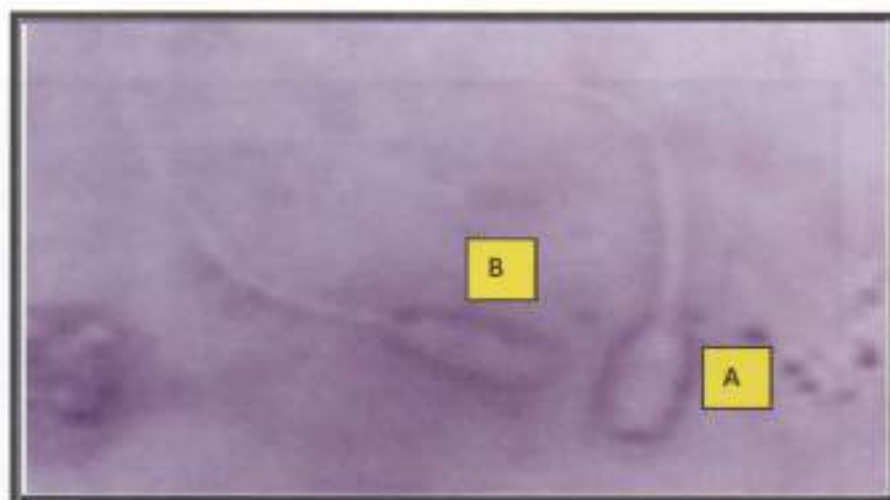
acid in cell causing at increasing of hydrogen ion exponent, causing metabolic process become to increase, and cause increasing of forming of ATP, so that will increase spermatozoa motility (Jones, et al., 1992; Garner and Hafez, 2000; Rigau, et al., 2002; Ford, 2006).

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 Supplementation 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 \pm 4,64 ^a
	60	78,60 \pm 3,45 ^a
3,0	30	87,20 \pm 2,79 ^b
	60	85,48 \pm 4,76 ^b
4,5	30	85,23 \pm 3,89 ^b
	60	83,20 \pm 6,34 ^b
6,0	30	76,50 \pm 9,81 ^c
	60	74,12 \pm 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 supplementation and treatment with acrosin supplementation 3,0; 4,5 and 6,0 μ 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 supplementation 3,0 μ gram at various incubations resulting mean of PE goats spermatozoa's viabilities percentages better from other treatment. This indicating that acrosin supplementation up to 4,5 μ gram in goat semen PE still give better effect to life of spermatozoa. acrosin supplementation 6,0 μ gram have shown degradation of

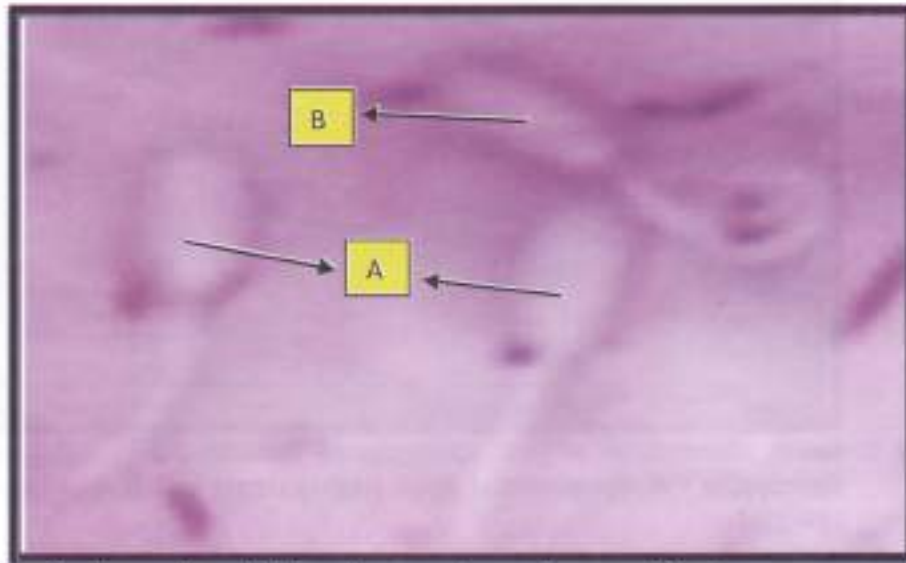
mean PE goat spermatozoa viability percentage. This is anticipated to relating of forming of lactic acid (Hafez, 2000).

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 Supplementation 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 abnormality 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

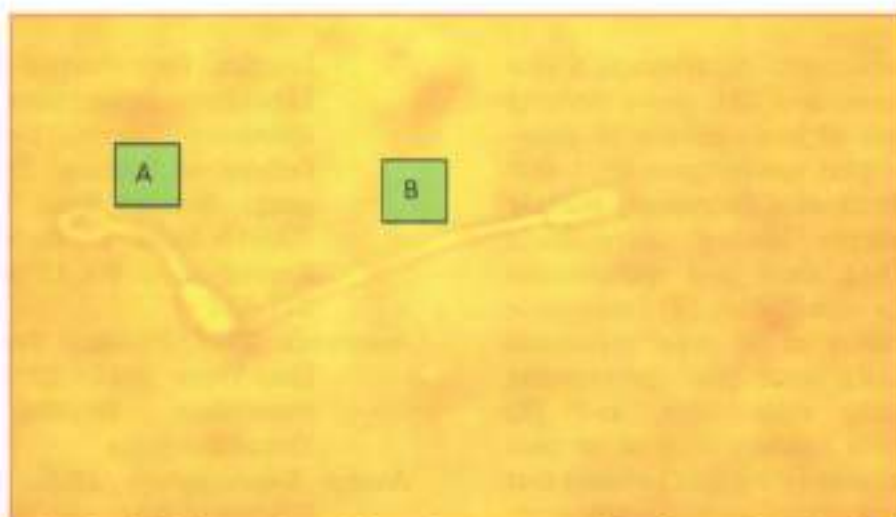
Result observation of PE goat spermatozoa abnormality with coloration of eosin negrosin observable at microscope phase contras of 1000 times expanded at picture of 2. Analysis result indicate that treatment without acrosin supplementation and treatment with acrosin supplementation dose of 6,0 μ gram at various incubations have an effect on not be real ($p>0,05$) to mean of PE goat spermatozoa abnormality percentage, while acrosin supplementation dose of 3,0 and 4,5 μ gram

at various incubations strippers have an effect on reality (p) to mean of PE goat spermatozoa abnormality percentage. The mean of PE goat abnormality percentage which obtained from this research less than 10. This indicate that process of handling of semen from relocation until done by supplementation technically have been put accross, so that secondary abnormality can be depressed as low as possibly.

Tables of 5. Influence of Acrosin Supplementanio 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 \pm 5,76 ^a
	60	45,87 \pm 4,49 ^a
3,0	30	46,44 \pm 4,19 ^a
	60	46,38 \pm 5,09 ^a
4,5	30	45,23 \pm 3,30 ^a
	60	45,44 \pm 2,36 ^a
6,0	30	45,87 \pm 2,45 ^a
	60	44,96 \pm 1,67 ^a

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

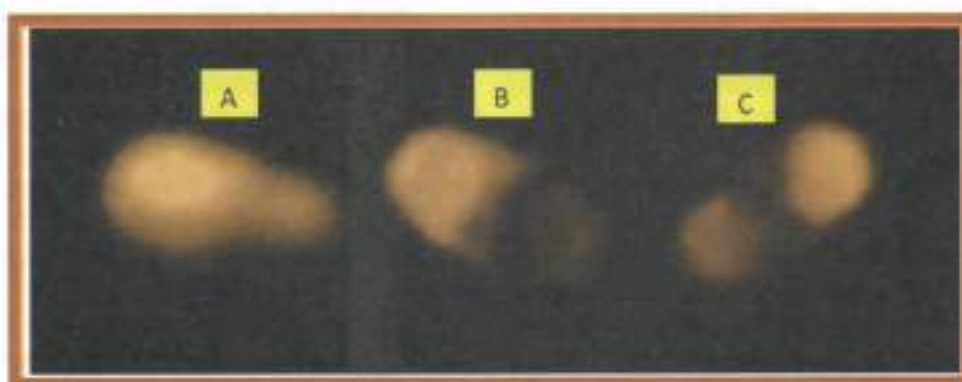


Draw 3. Result Observation of Goat Spermatozoa Membrane Integrity by Light Microscope Phase Contrasts, discription : A. spermatozoa with intake membrane (circle tail), and B. spermatozoa with damage membrane (tail straight). 400x

Tables of 6. Mean of PE goat Spermatozoa Capacitation Status Percentage after Acrosin Supplementation and long of incubation.

Periakuan		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 ^b	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 ^b	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 epifluorescence microscope (Bar = $\frac{1}{2}$ µ $\frac{1}{2}$ 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 fluorescence at goat spermatozoa head, that is (1) distribution fluorescence is same at membrane leading spermatozoa (draw 4.6A), show goat spermatozoa which non capacitation, (2) fluorescence concentration of at area acrosomal (draw 4.6B), show goat spermatozoa experiencing capacitation, and (3) fluorescence concentration of at post acrosomal area (draw 5.6C), shown that goat spermatozoa experiencing acrosome reaction. Result of luminescence of fluorescence at goat spermatozoa (draw 4.6.) is image of distribution Ca^{2+} at spermatozoa head membrane, like have been reported by Mattioli, et al (1996), Caul et al (1997) and ethic (2005).

CONCLUSION

Based on solution and result from this research is inferential as follows : Supplementation acrosin 4,5 μ gram can increase PE goat spermatozoa motility, Supplementation acrosin 6,0 μ gram can reduce PE goat spermatozoa motility. Supplementation acrosin 3,0 and 4,5 μ gram can increase viability, PE goat spermatozoa acrosome reaction and capacitation. Supplementation acrosin 3,0 and 4,5 μ gram can reduce non PE goat spermatozoa capacitation. Supplementation (addition) acrosin with dose of 4,5 μ 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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