The Effect of Combination Sweet Orange Peel Essential Oil with Trish Yolk and Streptomycin Extender on the Quality Boer Goat Liquid Semen

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Abstract: The purpose of this research is to know the quality of Boer Goat liquid semen after being given combination of sweet orange peel essential oil with streptomycin on tris yolk extender. The research used completely randomized design with 5 treatments and 5 replications. Treatment research is the addition sweet orange peel essential oil to tris yolk and streptomycin extender are 0%, 0,25%; 0.5%; 0.75% and 1%. Parameters were Motility, viability, Membrane Integrity, and Acrosome Integrity. Observations are made before and after equilibration. The results showed that the addition of sweet orange peel essential oil was significantly influenced ($P < 0.01$) to percentage of motility, viability, Membrane Integrity, and Acrosome Integrity liquid cement of Boer Goat. The best research treatment is the addition 1% sweet orange peel essential oil.

1 INTRODUCTION

The need for meat in Indonesia is very high because of the rising population, the awareness of the importance protein needs and the increase of people's income. To meet these needs may be done by livestock producers of meat.

Goat cattle is one effort that can be done to meet the demand of meat. Goats are small ruminants cattle that are kept by livestock farmers in the village. Goats have the advantage of being able to adapt in extreme conditions, resistant to disease, rapidly proliferate and proliferate so that goats are very potential to be developed (Mulyono and Sarwono, 2008).

To develop a goat farm can be done by improving genetic quality and population. Genetic quality of goats can be improved through crossbreeding with superior goats, such as the Boer Goats. The Boer Goats have the superiority of having a high body weight, fast growth and high litter size (Ginting and Mahmilia, 2008). Boer goat good quality still hard to find in Indonesia. It is necessary for the optimal use of the Boer Goat males.

Artificial Insemination is a reproductive technology that can be done to increase the population and genetic quality of livestock. Artificial Insemination has the advantage that the spread of goat livestock can be done cheaply, easily and quickly because the breeders do not need to buy and maintain the Boer Goat male.

Artificial Insemination program's success depends on the quality of liquid semen is used. Good quality semen is obtained from superior goats, as well as semen processing from shelter to dilution. The materials used in the manufacture of liquid semen must be considered. Selection of the type of semen extender greatly affects the quality of the resulting liquid semen. In goat semen extender used is still being developed. Tris yolk extender is very commonly used in the preservation and cryopreservation process of semen of various types of animals and livestock. Tris yolk extender are composed of: tris, citric acid, fructose and akuabidest (Rizal and Herdis, 2008).

The decreasing quality of Boer Goats liquid semen is caused by the development of bacteria contained therein. Bacteria enter the liquid semen from the shelter process and the manufacture of less hygienic semen. To inhibit bacterial growth can be accomplished by the addition of antibiotics, one of which is streptomycin (Rizal and Herdis, 2008). However, the addition of streptomycin is still lacking inhibit bacterial growth so we need a combination with other materials that contain antibacterial.

One of the ingredients that contain antibacterial is essential oil. Essential oils widely used fragrance chemical industry, adds flavor to beverages and

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foods. In the pharmaceutical and health industries are used as, antiinfectives, antibacterials, viruses and fungi (Lutony, 1994). Sweet orange essential oil contains the main components such as limonene, linalool which is toxic in bacteria (Fisher and Phillips, 2006).

Combining streptomycin and essential oils of sweet oranges are expected to minimize bacterial growth, improve the quality and survival of spermatozoa in frozen semen and increase the fertility rate of Boer Goats. Boer Goat liquid semen produced can be used in the development and improvement of the goat population through artificial insemination.

2 RESEARCH METHODS

2.1 Materials and Equipment

The research materials used are fresh cement of Boer Goat, streptomycin, Sweet orange essential oil and tris yolk extender, citric acid, fructose, 2% eosin, formolsaline, aquabidestillata, warm water (45-55 º C), HOST (Hipo Osmotic Swelling Test) and tissues.

The research tool used is 400 ml beaker glass, deck glass, tissue, stirrer, an artificial vaginal set, test tube, water bath, electric microscope, thermometer, dropper drop, object glass, cover glass, pH meter, bunsen burner, denominator , container cage and mat.

2.2 Methods

The research method used is a complete randomized design trial method using 5 treatments and 5 replications.
The treatment provided is:
P0 = Streptomycin + Essential Oils 0%
P1 = Streptomycin + Essential Oils 0.25%
P2 = Streptomycin + Essential Oils 0.5%
P3 = Streptomycin + Essential Oils 0.75%
P4 = Streptomycin + Essential Oils 1%.

2.3 Evaluation Parameters

Motility: Assessment of motility percentage is done by observing progressive moving spermatozoa forward. The evaluation was performed using a microscope that observed spermatozoa in eight different fields of view with 400 times magnification light (Toelihere, 1993).
Viability: Observations were made by using eosin staining on the sample (Toelihere, 1993). Live spermatozoa characterized by the head of spermatozoa which do not absorb the color, while the die is characterized by a head to absorb the color. The evaluation was performed using a 400 times magnification light microscope. Spermatozoa were observed at least 200.
Membrane integrity: The observations were performed on the integrity of the plasma membrane of spermatozoa using hypoosmotic swelling test method by mixing 0.1 ml of semen and 9.9 ml of hypoosmotic medium, then incubating at waterbath at 37 ° C for 30 minutes (Rodriquezgil et al., 1994). The evaluation was performed using a microscope at 400 times magnification.
Acrosome integrity: The observations were performed on the spermatozoa acrosomal whiteness of the head against the thick black spermatozoa by mixing 0.1 ml of semen and 9.9 ml a physiologic NaCl solution containing 1% formalin (Saacke dan White, 1972). The evaluation was performed using a microscope at 400 times magnification. Spermatozoa were observed at least 200.

3 RESULTS AND DISCUSSION

3.1 Research Result

The results of Boer goat semen research before and after equlibrasi by using a combination of streptomycin and sweet orange essential oil in the diluent can be seen in Table 1.

Table 1: Recapitulation of research results of semen quality of Boer Goats before and after equilibration.

		Observation	
Parameters	Treatments	Before Equilibration	After Equilibration
Motility	0%	67	69
	0,25%	70	72
	0,5%	71	75
	0,75%	73	75
	1%	75	77
Viability	0%	80	77
	0,25%	85	80
	0,5%	87	83
	0,75%	88	84
	1%	89	86

Table 1: Recapitulation of research results of semen quality of Boer Goats before and after equilibration (cont.).

Parameters	Treatments	Observation	
		Before Equilibration	After Equilibration
Membrane integrity	0%	72	70
	0,25%	77	75
	0,5%	79	76
	0,75%	80	77
	1%	83	81
Acrosome integrity	0%	68	65
	0,25%	72	70
	0,5%	74	72
	0,75%	75	74
	1%	77	76

Description: Different superscript on the column showed a highly significant difference (P<0.01)

3.2 Discussion

Boers liquid semen quality is a determining factor for the success of fertilization after artificial insemination. determinants of the quality of microscopically viable spermatozoa for artificial insemination ie motility, viability, membrane integrity percentage and the percentage of acrosome integrity**.**

3.2.1 Percentage Motility of Boer Goat Spermatozoa

The results of observational studies of fresh semen motility after preparation using tris diluent yolk and essential oil and observations after equilibrasi show different results each treatment. According to Hafez (1987), the motility of spermatozoa is one of the determinants of successful spermatozoa to reach the ovum of the fallopian tube and the simplest way of sperm assessment for artificial insemination.

The results showed the average observation of each motility treatment on all treatments meet the standards for use as liquid semen for Artificial Insemination because the above motility of 67% and the number has been eligible for Artificial Insemination. Sufficient semen requirements Artificial Insemination has a motility that is less than 40% (Evans and Maxwell, 1987).

The best spermatozoa motility that can be used and meet the standard in this research is on the addition of 1% essential oil as diluent and the higher the essential oil level given to the diluent will further decrease sperm motility either after dilution and after equilibration. The quality of semen will decrease if storage is not coupled with the right diluent (Hafez, 2000).

Hydroxynonenal is one of lipid peroxidation that can inhibit glycolysis and sperm motility. In addition to the damage caused by lipid peroxidation, decreased motility can also occur due to several factors White (1993). According Tounere (1993) factors that can decrease motility that changes pH Medium, osmotic pressure and the effects of electrolyte and non electrolyte. Decreased sperm motility may be due to high levels of essential oils used in diluents. Lipid peroxidation which occurs as a result of increasing oil content in the diluent is too large.

3.2.2 Percentage Viability of Boer Goat Spermatozoa

The immobile spermatozoa may not necessarily die so as not to absorb the color, whereas the interpretation of the moving and immobile base is considered immotile. At the spermatozoa are alive and moving, but there are defects in the cell wall, can absorb the color is presumed dead, while the other interpretation is considered not die Partodihardjo (1982)

The result of variance analysis showed that the effect of the addition of combination of streptomycin with essential oil of sweet orange as a diluent had a very significant effect (P <0.01) on the viability of spermatozoa both before and after equlibration. Further test results showed that the best viability was found in 1% addition of 89% before equlibration and 86% after equlibration.

The National Standardization Agency determines the quality of semen after the freezing process should show live spermatozoa (viability) of at least 40% (Anonimous, 2005). Decreased spermatozoa quality after equlibration caused by spermatozoa experience cold shock (cold shock) (Toelihere, 1993).

3.2.3 Percentage Membrane Integrity of Boer Goat Spermatozoa

An intact plasma membrane integrity of spermatozoa that was instrumental in the process of fertilization to the success of artificial insemination. Spermatozoa with intact membrane will retain hypoosmotic fluid inside the cell so that it does not suffer damage (Hafez, 2000).

The result of analysis of intact plasma membrane variety of Boer goat showed a very real effect (P <0.01) on the addition of essential oil as diluent. Viewed from the average value of the highest intact plasma membrane of Boer Goats at P4 treatment ie 83% before equilibrium and 81% after equilibration. Sweet orange essential oil serves as an antioxidant very useful for intact intact plasma membrane Boer Goat. This is in accordance with the opinion of Maxwell and Watson (1996) which states that the spermatozoa plasma membrane is rich in unsaturated fats and therefore susceptible to lipid peroxidation. The result of lipid peroxidation is the formation of lipid peroxides, which will react as free radicals and induce an autocatalytic reaction, resulting in the destruction of the plasma membrane (Sinha et al., 1996).

The results showed that the percentage of intact plasma membranes was also associated with viability of spermatozoa. Spermatozoa with a high percentage of live shows a high percentage of intact plasma membranes as well. Spermatozoa that have a high percentage of life indicate that the plasma membrane is still intact physically, so the organelles of spermatozoa cells will be protected, the need for food substances and ions for metabolic processes is available (Tambing et al., 1999).

Higher levels of sweet orange essential oil administration it will increase the percentage of intact plasma membrane. This may be due to a higher lactose concentration causing a change in osmotic pressure in the diluent toward the hypertonic. Hypertonic diluents indicate that the molecules or particles outside the cell are more than in the cell. As a result there is discharge of water from within the cell to thin out the molecules outside the cell, so that the cell will contract (Supriatna and Pasaribu, 1992).

3.2.4 Percentage Acrosome Integrity of Boer Goat Spermatozoa

Acrosome integrity role in the fertilization process to the success of artificial insemination. The changes that occur in acrosom are more often associated with fertility than sperm motility.

The results showed the highest percentage of intact acrosomal cap on P4 treatment after dilution of 77% and 76% after equilibration. The results showed that increasing the concentration of essential oils in the diluent will cause the average percentage of intact sperm acrosome covering the lower Boers.

The low percentage of intact acrosome hood and intact plasma membrane is associated with low percentage of motility, viability and intact plasma membrane. According to Salisbury and Van DeMark (1985) there is a physiological relationship between motility and the integrity of the plasma membrane and the survival of the spermatozoa. Plasma membrane damage will cause the loss of the necessary enzymes

4 CONCLUSIONS

The best combination of streptomycin and sweet orange essential oil with percentage of motility, viability, membrane integrity and acrosome integrity of Boer Goat's cement is at 1% level.

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