Influence of combination of bean sprouts extracts and tris-quail yolk on quality frozen semen of holstein friesian bull

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Abstract. The quality of frozen semen may decrease during the freezing and thawing due to ice crystals and free radicals. Efforts to improve the quality of frozen semen can be done by modifying the diluents and the addition of cryoprotectant compounds. The bean sprouts extract contains antioxidant compounds that can function as a cryoprotectant in the diluent. This study aims to determine the effect of addition and optimum concentration of bean sprouts extract tris-quail yolk in which can be used to improve the quality of frozen semen of Holstein Friesian Bull. This research consisted of 6 treatments (P) and 4 replications with Completely Randomized Design (CRD). P1 is tris-yolk quail with addition of 0.1%, 0.2% P2, P3 0.3%, 0.4% P4, and P5 0.5% of bean sprouts extracts. As for the control treatment (P0) is Tris-yolk quail without the addition of bean sprouts extract. The results showed that the addition of 0.3% of bean sprouts extract haves similar microscopic quality of frozen semen covering: motility, live sperm, acrosome cap, plasma membrane and intact abnormalities with the mean as follows 53.25%, 76.75%, 90.75%, 60.25% and 5.25% as normal as control. It can conclude that the combination of bean sprouts extract and tris-quail yolk can improve the quality of frozen semen of Holstein Friesian Bull

1. Introduction
Demand of milk in Indonesia increased by 14.01% over the period between 2002 and 2007. While milk production in Indonesia grew only 2% [5]. This condition is related to the number of dairy cow population and productivity of dairy cattle in Indonesia. According [6] The dairy cow population scattered in several provinces in Indonesia. Dairy cow population in 2009 in East Java, Central Java and West Java in a row, namely, 221 944, 134 821 and 114 588. While in the West Sumatra provincial dairy cow population in 2009 is 779 tails.

Padang Panjang is the central areas of the dairy farm in the province of West Sumatra. Population of dairy cows in Padang Panjang in 2014 reached 1,602 tails [7]. Milk production in West Sumatra province is still low at 700 ml / head / day [22]. Susu dairy cattle have been processed with various variants such as yogurt, pure milk, milk with a variety of flavors at Home Milk Padang Panjang.

the milk production of domestic low caused high imports of milk in Indonesia. this condition can lead to self-sufficiency and food sovereignty especially milk increasingly short of expectations, and the potential compliance milk intake is highly dependent on market conditions exporter [6].
The low production of milk in the country due to some problems among them, the low population of dairy cows with productivity levels high 11 liters/day [6]. for that we need an effort to increase the population of dairy cows in the country with reproductive technology.

One of reproductive technologies that can be used is the Artificial Insemination (AI). AI reproductive technology can optimize the function of the male seed. However, AI implementation in Holstein Fresian dairy cows (FH) is still less widely applied in the province of West Sumatra. It is facing many obstacles, one of which is the quality of frozen semen produced [2].

One of the factors that determine the success of the manufacture of frozen semen is diluent with various additions cryoprotectant. Cryoprotectant is a component that must be present in the preservation medium that serves to minimize physical stress and chemical sperm cells resulting from the process of cooling, freezing and thawing [14]. The addition of the diluent cryoprotectant may protect spermatozoa from the lethal effects and damage during the freezing process. For that, they need to do development on adding a cryoprotectant compound in the dilution of frozen semen.

Cryoprotectant is a compound that is both extracellular and intracellular protection for cement ternak. Krioprotektan extracellular example is a saccharide group, and egg yolks. While the intracellular cryoprotectant example is the antioxidant compound and glycerol. There are currently frozen semen has been produced by some of Artificial Insemination Centers using the Tris-yolk [2].

Research on the effects of antioxidants compounds and in thinning of frozen semen has been conducted since 1993. According to [14] β-carotene with a concentration of 0.02% were able to improve the quality of frozen semen Sheep. Research results show that β-carotene concentration of 0.02% worth 50.55% and 56.78% on motility and sperm alive. [6]. At [1] suggest that the addition of the diluent into the onion extract AndroMed® 2% during storage cold temperatures affect the quality of frozen semen motility goat with a value of 63.75% ± 5.62%. The addition of onion extract into diluent AndroMed® with a concentration of 2% at room temperature significantly affect the quality of frozen semen Peranakan Etawah goat. Frozen semen motility on hour-1, 2 and 3 are as follows: 62.50 ± 6.3%, 5.6% ± 61.25 and 50.00 ± 0.0%. However, the addition of antioxidant compounds in the diluent showed varying results that still need to do more research to get the results more better [18].

Natural herbs have antioxidant compounds, one of which is the green beans. Bean sprout germination green beans are commonly consumed as a vegetable. According to [8] vitamin that can be found in bean sprouts is vitamin C, thiamin, riboflavin, niacin, thenik panto acid, vitamin B6, pholate, choline, β-carotene, vitamin A, vitamin E (α-tocopherol) and vitamin K. Moreover, in an extract of bean sprouts have a micro minerals, macro and essential amino acids.

There is no information about the use of the extract as an additive bean sprouts antioxidant compounds in Tris-yolk quail to improve the quality of cattle frozen semen FH yet. The bean sprouts extract have a lot of antioxidant compounds that can increase the quality of frozen semen FH cow. This study aims to determine the optimum concentration of the extract of bean sprouts on a quail egg yolk Tris diluent to improve the quality frozen semen of FH.

2. Materials and Methods

2.1. Materials

Frozen semen diluents consisting of trisolution buffer and quail egg yolks with the addition of bean sprouts extract as a treatment. Tris buffer solution consisting of: Tris (hydroxy-methyl-amino-methane) 2.98 g, 1.65 g Citric Acid, Fructose 2 g egg yolks 20 ml, penicillin 100,000 (IU), Streptomycin 100 mg, aquabidest 100 ml [24]. Materials for the treatment of bean sprouts extract 100 ml. Live sperm count test solution is a solution of eosin 2%. The solution used in the test intact acrosome hood is a physiological saline containing 1% formalin [14]. The solution used in the test intact plasma membranes are made with the composition of HOS solution 1,352g and 0,735g fructose Na-citrate in 100 ml of distilled water [21].
2.2. Methods
Frozen semen manufacturing process follows the standard work UPTD BIB Tuah Sakato Payakumbuh. The initial stage in the creation of shelters and frozen semen is macroscopic analysis to test the feasibility of fresh cement into frozen semen. Frozen semen manufacturing process starts from the shelter, dilution, filling and sealing, equilibrasi, freezing, storing and thawing.

Evaluation sperm cow quality as macroscopic and microscopic observation. Macroscopic examination includes the volume, color, odor, pH and viscosity of the sperm. The microscopic examination are: sperm motility, percentage of survival, abnormality, hood acrosome intact, and intact plasma membrane.

To calculate the number of spermatozoa, using the Improved Neubauer. As for seeing and counting the number of abnormal sperm (the morphology of spermatozoa), using eosin staining method. The Data Analyzed by ANOVA (Analysis Of Variance) and Duncan's New Multiple Range Test.

3. Result and Discussion
Semen Holstein Friesian cows housed in the morning. Ejaculated semen obtained is then evaluated by observing the macroscopic and microscopic characteristics. Macroscopic and microscopic evaluation of fresh semen is useful to determine the viability of frozen semen is processed into semen. The observation of fresh semen quality Friesian Holstein cows contained in Table 1.

| No | Treatment % | Treatment | Motility | Number of live sperm | Hood acrosome intact | Intact plasma membrane | Abnormalities |
|----|-------------|-----------|----------|----------------------|----------------------|------------------------|--------------|
| 1  | P0          | 53.75<sup>a</sup> | 70.75<sup>a</sup> | 91.00 | 50.00 | 7.25<sup>a</sup> |
| 2  | P1          | 49.50<sup>a</sup> | 57.00<sup>a</sup> | 76.50 | 59.00 | 12.00<sup>a</sup> |
| 3  | P2          | 49.00<sup>a</sup> | 50.50<sup>a</sup> | 82.00 | 58.50 | 6.26<sup>a</sup> |
| 4  | P3          | 53.25<sup>a</sup> | 76.75<sup>a</sup> | 90.75 | 60.25 | 5.25<sup>a</sup> |
| 5  | P4          | 45.50<sup>ab</sup> | 59.25<sup>ab</sup> | 83.25 | 39.50 | 9.75<sup>a</sup> |
| 6  | P5          | 44.75<sup>ab</sup> | 45.50<sup>ab</sup> | 79.75 | 57.75 | 15.50<sup>b</sup> |

Description: number followed by the same letter in the same column are not significantly different at P <0.05%.
Microscopic spermatozoa is done by using a phase contrast microscope with a magnification of 400 x. The observation of live sperm in Figure 1. The observation of intact acrosome hood in Figure 2. The observation of intact plasma membrane in Figure 3. The observation of abnormal spermatozoa and normal spermatozoa in Figure 4.

Figure 1. Sperm life, not eosin stained (a) and dead spermatozoa, eosin stained (b).

Figure 2. The hood acrosome intact (c) and the hood is not intact acrosome (d).

Figure 3. The plasma membrane intact (e) and incomplete (f).

Figure 4. Spermatozoa small head (g), the two head spermatozoa (h), tail is crooked (i) and normal spermatozoa (j).
Based on statistical tests on sperm motility showed that the highest rates are P0 is 53.75%. Sperm motility treatment P1, P2, and P3 are not significantly different at a further test to P0. The addition of bean sprouts extract significantly different in treatment P4 and P5 are 45.50% and 44.75%. Results of recovery rate cattle FH was 57.53 ± 1.74% [20]. Motility after six days of preservation in tris diluent yolk was 40.83% while the carrot juice 30% ie 30.83% [10]. According to [9] motility Limousin beef stored at cool temperatures in the yolk diluent and guava juice for 24 hours was 33 ± 5.63%; 36.5 ± 4.89%; and 30 ± 4.41%. Meanwhile, according to [11] the results of sexing sperm motility after thawing is 52.73% 43.78% sperm X and Y sperm motility degradation affected by a high concentration of sugar in the diluent. The content of glucose and fructose in a row bean sprouts are 4% and 0.4%, whereas the sucrose content of 3.2% [19]. In the solution buffer containing 2 grams of fructose, which means that there are 2% of fructose in the solution. High sugar concentration in the diluent will increase osmotic pressure diluent solution that cannot be adapted well by spermatozoa. This will disrupt flow biokemik processes normally in cells. According to [16] the addition of as much as 0.2% lactose liquid cement produced with a lower quality than at 0.1%. This shows that the addition of lactose 0.1% is the optimum concentration in Tris against liquid semen quality Garut sheep were stored at 5°C.

The sperm motility was also influenced by the cholesterol in egg yolk quail. Quail egg yolk contains high cholesterol which is 2139.17 mg / 100g [5]. The content of the diluent high HDL can inhibit respiration and motility [12]. Biochemistry abnormal process and hamper respiration of spermatozoa also affect the number of live sperm after thawing. Dead spermatozoa spermatozoa are marked with red color after exposure to eosin 2%. Live sperm cells do not absorb the dye whereas dead spermatozoa will absorb the dye due to increased cell membrane permeability as dead [25]. Based on the statistics indicate that the bean sprouts extract concentration effect on the number of live sperm. The addition of bean sprouts extract to a concentration of 0.4% was not significantly different to the number of live sperm P0. However, the addition of 0.3% sprouts extract the optimum concentration of the number of live sperm with the average of 76.75%. According to [17] the percentage of spermatozoa in Egg Yolk Tris 33.3 ± 2.64. Meanwhile, according [3] the number of live sperm in the egg yolk tris diluent is 65.93%.

The number of live sperm is affected by the amount of energy in the diluent and plasma membrane integrity of sperm cells. Based on observations, adding bean sprouts extract 0.3% is also an optimum concentration with the average of 60.25% of the plasma membrane intact. According to [13] plasma membrane damage can cause the loss of the necessary enzymes in the metabolic processes so that no energy is generated and decreases the motility and viability of spermatozoa.

Average hood acrosome intact based observations showed a decrease in quality by increasing concentration of extract of the bean sprouts in the diluent. However, based on further testing of the sprouts extract concentration difference no significant effect on the decline in the quality of acrosome intact hood. The results showed that the optimum quality intact acrosome hood is P3 is 90.75%. Meanwhile, P5 is the lowest quality is 79.75%.

Acrosome integrity of the hood associated with the plasma membrane integrity of spermatozoa. Plasma membrane of intact cells protect the acrosome vesicle at the tip of the head of the physical destruction, so that acrosome vesicle remain intact and acrosome intact hood value increased (Rizal, 2005). Meanwhile, according to Holm and Wishart in [3] setting function of mammalian and non-mammalian spermatozoa such as the acrosome and motility are affected by a decrease in the internal pH of spermatozoa.

The addition of the diluent bean sprouts extract was not significantly different to the plasma membrane intact spermatozoa. However, the increased concentration of bean sprouts extract up to 0.3% can increase the average results of plasma membrane intact spermatozoa. Mean plasma membrane intact P3 is the highest is 60.25%. Treatment can control kulitas retain intact plasma membrane with an average 50.00%. These results show the same state at the research [21] using a combination of coconut water and tris-yolk quail can also increase the percentage of plasma membrane integrity of spermatozoa. This is presumably because the content of LDL in the quail egg...
yolk can protect sperm plasma membrane. Amirate, et al. in [12] states that the content of low-density lipoproteins (LDL) protects sperm from damage during preservation while high density lipoproteins (HDL) may inhibit respiration and motility spermatozoa. Selain, the addition of bean sprouts extract up to 0.3% tend to increase plasma membrane intact spermatozoa. The observation of intact plasma membrane can be seen in Figure 4. This is presumably because the content of vitamin as an antioxidant contained in the bean sprouts extract can protect the sperm plasma membrane of the physical damage caused by free radicals. Extract sprouts contain vitamin A, vitamin C, which is useful as an antioxidant in the preservation process. According to [14], vitamin E, vitamin C, β-carotene, glutathion and sintein a class of antioxidants that can break the chains of free radical reactions.

Treatment sprouts extract significantly different additions to the quality of sperm abnormalities. Spermatozoa abnormalities increases with the concentration of extract of bean sprouts. Based on observations of abnormalities P3 lowest was 5.25%, lower than 7.25% in the control treatment. The observation of abnormal spermatozoa can be seen in Figure 5. While the highest average is the addition of bean sprouts extract 0.5% ie 15.50%. According to [17] abnormality below 20% of a of frozen semen post-thawing does not cause a decrease in fertility in artificial insemination treatment. According to [4] the lowest abnormalities during 8 days storage in the diluent 75% egg yolk and 25% coconut water that is 24.15 ± 3.00. Meanwhile, according to [9] abnormality of diluent (CEP-2 + 20% Egg Yolk) showed the best results during storage 0 hours - 24 hours each of (11.98 ± 0.91%; 12.14 ± 1.08%; 12.41 ± 1.25%; 12.8 ± 1.10% and 15.45 ± 1.80%).

Based on further testing showed that the addition of the diluent bean sprouts extract up to 0.4%, not significantly different with sperm abnormalities. Decreased spermatozoa quality bean sprouts extract significantly with the addition of 0.5%. Abnormalities in spermatozoa abnormalities can be divided into primary, secondary and tertiary. Some of the primary abnormality that can be found from the research is the double head and microsephalus. Secondary morphological abnormalities of spermatozoa abnormalities that occur during sperm pass through the reproductive tract. Secondary abnormalities that can be found from this study is a simple bent tail, it is similar to that found by [23]. While the tertiary abnormality is an abnormality of spermatozoa due to treatment and handling during storage [2]. Another possibility according to [9] the manufacture preparatus less precise cause damage in spermatozoa such as the tail and the head severed. Tertiary abnormality that can be seen from the research is the tail broken off and a circular motion on the spermatozoa. Circular motion on spermatozoa allegedly due to the occurrence of shockcool due to less solubility glycerol.

The results of statistical analysis showed that adding extracts of bean sprouts in a quail egg yolk tris diluent affects the quality of microscopic post-thawing of frozen semen. Although each treatment was not significantly different to the hood intact acrosome and plasma membrane intact, but the addition of bean sprouts extract can maintain the quality of frozen semen. This indicates that the antioxidant compounds in the extract of the bean sprouts can reduce oxidation and free radicals when the thawing of frozen semen.

Based on further test, the addition of an optimal sprouts extract can be used in a quail egg yolk tris diluent is P3 is 0.3%. The addition of 0.3% bean sprout extracts can maintain the quality of frozen semen microscopic include: motility, the number of live sperm, the acrosome intact hood, and abnormality of plasma membrane intact with a mean respectively as follows (53.25%, 76.75%, 90, 75%, 60.25% and 5.25%).

4. Conclusion
Based on the results obtained in this study it can be concluded that the addition of the diluent sprouts extract tris-yolk quail can maintain the quality of frozen semen after cow Holstein Freisian thawing.

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