ORIGINAL ARTICLE

Effect of Extract Pumpkin Seed (*Cucurbita Moschata*) on Post-thaw Variabels of Local Rooster Semen

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ABSTRACT

Introduction: Semen conservation is one of the conservation effort in preserving germplasm. The frozen semen is generally used to extend the life capacity of semen. Before freezing process, the semen must be given cryoprotectant to prevent ice crystal formation and stabilize spermatozoa plasma membrane. However, the chicken sperm is more sensitive to the freezing process and their quality post thawing is also relatively low compared with the mammalian species. Thus, the material of cryoprotectant for semen is very interesting to study. This study aimed to improved quality of post thawing spermatozoa by giving extracted pumpkin seeds in domestic rooster. **Methods:** A total of twenty male domestic rooster aged one year oldwere divided into five treatment groups (T0, T1, T2, T3, and T4). Different level of extract pumpkin seeds (0%, 10%, 15%, 20%, and 25%) dissolved with cryoprotectant in frozen storage. Motility of sperm, viability, and sperm abnormalities were assessed before and after freezing cryopreservation. **Results:** The result demonstrated that the motility of sperm and viability significantly affected ($p<0.05$) for each treatment. The highest mean value after cryopreservation with the T2 concentration (motility of sperm : 36,5475 ± 1,46700) and (viability : 65,9600 ± 1,71416). Similarly, spermatozoa abnormalities were lower after cryopreservation with the T2 concentration (24,5000 ± 2,08029). **Conclusion:** The research indicate that supplementation of extract Pumpkin seed in cryoprotectant could significantly improve quality of post-thaw spermatozoa, therefore it could support productivity of domestic rooster in tropical area.

Keywords: Chicken, Cryoprotectant, Pumpkin seed, Spermatozoa

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INTRODUCTION

Indonesian local chicken as well as exotic to be germplasm. The local chicken (non-breed chicken) more resistant to the disease than "breed chicken" (1). It has 44,07% AA genotype (resistant to AI virus), 38,69% AG genotype (resistant/sensitive) and 17,24% GG genotype (sensitive) by testing the Mx gene. At present, there are at least 34 type of local chicken in Indonesia. Thus, the most popular and reared almost thoroughot the entire country is kampung chicken (2,3). However, it has slow sexual maturity, low productivity, and relatively expensive price because the high demand which not offset by increased production. Increasing the quality and quantity of sperm is one of the problem solving in the context of developing national farms and conserve the plasmagerm (4).

Artificial insemination (AI) in the poultry is the method of choice to increase poultry production, genetic improvement and control of veneral diseases (5-7). The Successfull of the method depends on strain of chicken, age of chicken, semen quality, diluent, dose of sperm, and storage of semen (8, 9). Both of Fresh and cryopreservation semen can be used for AI in the poultry (10-12). Additionally, the cryopreservation semen can protect the plasmagerm in danger of loss. However, The chicken sperm is more sensitive to the freezing process and quality post thawing is also relatively low compared with the mammalian species (13, 14). The freezing and thawing of spermatozoa can induces several forms of cellular lesion. These lesion have been attributed to cold shock, extreme osmotic change, ice crystal formation and reactive oxigen species (ROS) (15).

The chicken semen contains a variety of unsaturated fatty acids which are oxidized during storage generate ROS. Oxygen species are very active at the cellular level resulting in damage to the spermatozoa. Spermatozoa are susceptible to lipid peroxidase by free radicals

such as hydrogen peroxide, superoxidic anions, and hydroxyl radicals which ultimately cause damage to the structure of the spermatozoa membrane (16). Prevention of ROS accumulation by administering antioxidants which can prevent free radical oxidation reactions against lipid oxidation (17). The pumpkin seed is a rich natural source of antioxidant. Pumpkin seeds contain tocopherols, carotenoids, and flavonoids. These three compounds have secondary antioxidant activity and function as intracellular antioxidants that can prevent the peroxidation of unsaturated fatty acids in cells (18).

MATERIALS AND METHODS

A total of twenty male domestic rooster aged one year old were used as sampel divided into five treatment groups (T0, T1, T2, T3, and T4). Different level of pumpkin seeds extract (0%, 10%, 15%, 20%, and 25%). Cemen were collected twice a week by abdominal massage method, then evaluated for volume, color, pH, consistency, concentration, motility, viability, abnormality. Good quality semen (70% progressive motility) is then made into one in a sterile test tube. Chicken semen is diluted with diluent solution that has been prepared, then evaluated the motility and live spermatozoa. Bassal diluent / diluent semen solution was prepared by mixing 3.07 g of Tris, 1.64 citric acid, and 1.26 g of fructose in 100 ml of distilled water containing 5% glycerol 20% egg yolk. In the treatment, the addition of ethanol extract of pumpkin seeds in various doses. The semen is then packed in a ministraw (volume of 0.25 ml / straw) and the straw tip is covered with polyvinyl chloride (PVC) powder. Semen diluted with cryoprotectant was re-evaluated macroscopically and microscopically, after that it was quantified at 5°C for 60 minutes and cooled by placing straw straws on a special rack which was placed 10 cm above the surface of liquid nitrogen vapor for 4 minutes, then immediately inserted in liquid nitrogen (temperature -196°C) for 24 hours. Thawing is done in a 37°C water bath for 30 seconds. Microscopic evaluation is done after equilibration and after thawing includes motility and viability spermatozoa.

Sperm concentration was calculated by a spectrophotometry. Sperm motility was measured as a percentage of progressive sperm motility and observed by manual microscopic under 100Ч and 400Ч. Percent viability was assessed by eosin–nigrosin staining. Minimum of 200 sperms were counted on each slide for the calculation of live and dead sperms as well as to note the number of abnor¬mal sperms per sample (19,20). The data were analyzed by Analysis of Variance (ANOVA) with Turkey's post hoc test using SPSS version 20.00 software.

RESULTS

The mean of semen quality from fresh native chickens, both macro and microscopic showed a normal range. Microscopic evaluation of fresh semen shows the volume of chicken semen with the means value 0,83 ± 0,01 ml, pH (7,2 ± 0,00), sperm concentration (4,28 ± 0,22 x 10^9 cell/ml), motility of sperm (93% ± 0,01), the percentage of sperm viability (90% ± 0,5) and means value of spermatozoa abnormalities 5,5% ± 0,01 (Table I).

Table I : Domestic rooster of Fresh Sperm quality

Characteristics	Average ± SD
Volume (ml)	0,81 ± 0,01
Colour	Specific
Consistency	viscous
Smell	Specific
pH	7,2 ± 0,00
Motility of mass	+++ (3) Very good
Motility of sperm (%)	93 ± 0,01
Concentration (10^9cell/ml)	3,68 ± 0,22
Viabilitiy (%)	90± 0,5
Sperm Abnormalities (%)	5,5 ± 0,01

Sperm quality of rooster post dilution before freezing cryopreservation

The results of the sperm quality test after dilution before freezing cryopreservation showed that there was a significant decrease in the sperm quality. Dilution of sperm with each treatment has a significant effect ($P < 0.05$) on the motility and viability of spermatozoa. Motility and viability of spermatozoa in T2 were seen the best among the other treatment with means value of sperm motility 68,1415%± 1,96157, viability of sperm (71,4400% ± 1,84962), and means value of spermatozoa abnormalities 16,8667% ± 1,2794 (Table II).

Sperm quality of rooster post Thawing

The result of post thawing sperm quality test showed a significant decrease. Post-thawing had a significant effect on sperm motility in each treatment. The best results were shown in T2 with means values motility of sperm 36,5475 %± 1,46700. The viability of spermatozoa between T1 and T3 was not significantly different but significantly different from T2. The best results of post-thaw viability of sperm were found in T2 (65,9600% ± 1,71416). While the abnormalities of sperm in T2 and T3 were not significantly different, but the best results were in T2 (24,5000% ± 2,08029) (Table III).

Table II : Means of Spermatozoa quality of domestic Rooster Post Dilution

Characteristics	Average ± SD				
	T0	T1	T2	T3	T4
Motility of sperm (%)	60,6417^{b} ± 0,91022	65,6811^{d} ± 0,58708	68,1415^{e} ± 1,96157	61,8652^{c} ± 0,67886	58,4200^{a} ± 0,82570
Viability (%)	66,4510^{b} ± 1,74635	69,1367^{d} ± ,88998	71,4400^{e} ± 1,84962	67,6900^{c} ± 1,32492	63,8267^{a} ±1,23538
Sperm Abnormalities	20,6000^{b} ± 1,1017	17,2500^{a} ± 0,5686	16,8667^{a} ± 1,2794	17,0000^{a} ± 1,2865	17,3667^{a} ± 1,0662

Means with different superscripts in a row differ significantly (P<0.05). SD = Standart deviation

Table III : Means of Spermatozoa quality of domestic Rooster Post Thawing

Characteristics	Average ± SD				
	T0	T1	T2	T3	T4
Motility of sperm (%)	29,8283^{b} ± ,43215	34,0267^{d} ± 1,80521	36,5475^{e} ± 1,46700	32,8449^{c} ± 4,01676	27,3592^{a} ± 1,69287
Viability (%)	61,0900^{b} ± ,66920	62,8367^{c} ± ,94485	65,9600^{d} ± 1,71416	62,3300^{c} ± 1,61505	58,3333^{a} ± 1,39786
Sperm Abnormalities	37,2133^{d} ± 2,75477	27,3000^{b} ± 1,51839	24,5000^{a} ± 2,08029	25,2333^{a} ± 2,52823	30,5567^{c} ± 3,88309

Means with different superscripts in a row differ significantly (P<0.05). SD = Standart deviation

DISCUSSION

In this study, the means of semen from fresh native chickens showed a normal range. This indicates that the domestic chickens used have healthy spermatozoa, which can be used for artificial insemination and frozen storage (semen conservation). The means of pH value of fresh semen (7,2 ± 0,00) indicates a good sperm metabolism. The pH of semen determines the life status of spermatozoa. The low and high pH will make spermatozoa die faster (21). The fresh semen of sperm morphology results more than 85% of the sperm were alive and normal.

However, the results of the sperm quality test after dilution showed that there was a significant decrease in quality compared with fresh semen. T2 showed that the best result of motility, viability of sperm and sperm abnormalities. The addition of dilution supplements with the extract pumpkin seed which serve as antioxidant, and mineral Zn which is in pumpkin seed has a direct impact on alive, motility and viability of sperm (22). T4 showed the motility and viability of sperm have decreased significantly but their values can be used artificial insemination. This is because pumpkin seeds also contain tannins and cucurbitine which are derivatives of terpenoid that have low dose of antihelmintic (23). In contrast to in vivo applications, increasing the dose of pumpkin seeds given in vivo resulted in increased motility and viability of sperm, as well decreased number of sperm abnormalities in native chickens (24).

Post-thawing had a significant effect on sperm motility in each treatment. The freezing semen at -196°C will decrease sperm motility because 60% of poultry sperm organelles suffered irreversible damage after cooling, freezing and thawing process (25). Lipid peroxidation reactions can be inhibited by the addition of antioxidants, which are substances that can bind free radical compounds. Antioxidant include phenolic acids, flavonoids, polyphenols, β-carotene, vitamin C, vitamin E, and lycopene (26). The T2 showed that the best result of motility, viability of sperm and sperm abnormalities post-thaw. The addition of 15% pumpkin seed extract to the diluent which as an antioxidant (β-carotene, vitamin C, and phenol). Phenol is a chemical compound that has an aromatic ring with a hydroxyl (-OH) group. Phenol can reduce free radical chain reactions that occur in the cell (27).

CONCLUSION

The conclusion of this study is the addition of pumpkin extract to the diluent can improve the quality of spermatozoa after dilution and post thawing. The best dose according to this study is the addition of 15% pumpkin seed extract to the diluent. Doses above 15% cause decreased motilty and viability of spermatozoa after dilution and post-thawing.

ACKNOWLEDGEMENT

The authors are thankful to the Rector of Universitas Airlangga and Faculty of Vocational Studies at the Universitas Airlangga, Indonesia, for proving all the necessary funds under the research grant 1408/UN.3/2019.

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