Sperm Quality of Hybrid Chicken Affected by Propolis, Honey, or Royal Jelly as Organic Diluent Materials and Storage Periods during Sperm Preservation

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ABSTRACT
The increasing population programs of cross-breeding chickens (hybrid chickens) need to be supported by breeding strategies that depends on artificial insemination programs (AIP). Sperm diluent is one of the important factors that support the survival of spermatozoa during the implementation of AIP. For this purpose, an effective and efficient diluent to maintain the quality of chicken sperm in an adequate period time is needed by chicken breeders. The present study aimed to determine the effect of adding organic diluent material (propolis, honey, or royal jelly) and storage time during sperm preservation on macroscopic and microscopic quality of hybrid chicken sperm. The results of the study were expected to be useful to provide an alternative organic diluent for poultry sperm supporting the program of artificial insemination especially in the development of hybrid chicken populations resulting by cross-breeding with local chickens. The experiment was designed by a completely randomized factorial design consisting of 2 factors: differences in the types of organic diluent materials (OD) and storage time (S). The type of organic diluent materials was propolis organic matter, honey, and royal jelly. Chicken sperm was stored with different storage periods: 0, 12, 24, 36, and 48 hours at room temperature. The result of the present study indicated that different organic diluents of chicken sperm resulted in a highly significant difference ($P<0.01$) in all micro and macroscopic parameters except the total of sperm abnormality. Honey as sperm diluents for hybrid chicken sperm was able to maintain normal sperm concentration and viability up to 24 hours of storage period at room temperature compared to propolis and royal jelly treatments. Honey was able to maintain the concentration of total sperm of up to 36 hours of 109.67×10^7. The different treatments of organic diluents caused very significant differences in all micro and macroscopic parameters except on sperm abnormality. However, either honey, propolis, or royal jelly reduced total abnormality mortality of hybrid chicken sperm. It was concluded that honey was able to maintain sperm quality, concentration, and sperm viability of hybrid chickens better than propolis and royal jelly.

Keywords: *hybrid chicken, sperm, preservation, organic diluent*

1. INTRODUCTION

The rooster (F1) resulted from crossbreeding program between selected local chickens and Brahma chickens and it is called ALOBRA chicken produced by the Laboratory of Animal Science (LLP) and Research Center of Aceh Cattle and Local Animal-University Syiah Kuala. Selected local chickens were native chickens that have better immunity than origin chickens. However, free-range chickens have a fairly low production due to simple maintenance management [1]. On the other hand, Brahma chicken has unique characteristics such as slow movement, pea-shaped comb, growing feathers around the legs, dominant has

white feathers, male weight 4.56-5.50 kg and female 3.65-4.33 kg. In accordance with the crossbreeding program, it is expected that the results of the F1 Alobra derivative chickens will be able to produce offspring that are better genetically, phenotypically and productive.

Hybrid chicken produced by crossbreeding program which used different breeds was greatly influenced by: mastery of insemination technology, control on the selection process and health quality of male and female chickens. Artificial insemination has many advantages for maximizing the use of male animals and preventing disease transmission [2]. In general, chicken spermatozoa survive for 30-45 minutes at room temperature and it is influenced by the diluent used during storage and sperm quality [3]. A good sperm diluent is a diluent that can provide nutrients for spermatozoa during storage that allow spermatozoa to move forward progressively. Sperm diluent is a non-toxic material, protects spermatozoa against cold shock, as a buffer or buffer preventing pH change, maintains osmotic pressure and electrolyte balance, and contains antibiotics that reduce bacterial growth [4].

In the process of dilution and storage of sperm, fructose and glucose act as a source of energy for spermatozoa [5]. Propolis, honey, and royal jelly have content that can meet these needs. Various studies have used honey to perform sperm dilution as applied to the spermatozoa of Etawah Goat, comet fish [6, and Catfish [7]. However, until now the effect of adding propolis, honey, and royal jelly to dilute poultry spermatozoa has not been widely reported. This research aimed to determine the effect of increasing the concentration of propolis, honey, and royal jelly on NaCl diluent and storage time of semen on the motility and viability of spermatozoa of F1 rooster crosses between local chickens and Brahma chickens (ALOBRA) stored at room temperature.

2. MATERIALS AND METHODS

This research used a factorial completely randomized design method which consisted of 2 (two) factors, namely the type of organic matter (P) and storage time (A). The organic matter factor (P) consisted of 4 treatments, namely control in the form of NaCl diluent, propolis, honey, and royal jelly, with each organic material added with NaCl. The time factor (A) was storage time, namely 0, 12, 24, 36, and 48 hours at room temperature (25º-29ºC). Each treatment has 3 replications. Sperm was collected from individual chickens. Sperm collection was carried out using the total sperm collection method from 4 roosters of each treatment. The roosters used were 8-9 months of age, in good health and body weight ranging from 2.85-3.0 kg. All roosters were reared in intensive cages equipped with feeders and drinkers.

The diluent used was NaCl with a concentration of 0.9% and added organic materials such as propolis, honey, and royal jelly with different doses. The ratio of sperm volume and diluent is 1:5 [8]. The used are physiological NaCl (0.9%), propolis, honey, and royal jelly.

The technique of collecting chicken sperm was done by massaging until the chicken experienced an erection, which was characterized by stretching of the chicken's body and papillae sticking out of the cloacal proctodeum. The sperm that came out was immediately accommodated using a test tube [9]. Then the sperm that came out were treated and measured for macroscopic and microscopic evaluation through storage times of 0, 12, 24, 36, and 48 hours. The parameters observed were macroscopic and microscopic qualities of sperm. Macroscopic measurements were volume, color, smell, and pH. Microscopic measurements were concentration, viability, motility, and abnormality. The color of the sperm is evaluated directly when the sperm is collected in the test tube. The smell of the sperm was detected by smelling the open part of the test tube and shaking it slightly so that the aroma could be smelled perfectly and then pH was measured by a pH measuring device. Microscopically evaluated as follows haemocytometer was used to calculate the total concentration of sperm with a dilution of up to 200 times; i.e., using a pipette erythrocytes sperm were taken up to the 0.5-line mark. Then the dilution was added up to the 101 marks so that 200 times the dilution was obtained. Calculations are carried out through the Neubauer counting chamber by counting 5 boxes from the 6 available boxes and then multiplying by 109 [10]. Sperm viability was obtained by dividing the live spermatozoa into the total number of spermatozoa.

The movement of individual sperm was observed by a microscope, 40 x 10 for individual movement [11]. There are 3 movements of spermatozoa, namely progressive movement (forward movement), rotational movement (rotating movement), and oscillator or convulsion without forwarding movement or positional displacement [12]. Sperm motility assessment was obtained by dividing the motile spermatozoa into the total number of spermatozoa. Abnormality assessment was carried out by staining spermatozoa to evaluate the number of live and dead spermatozoa, as well as to evaluate spermatozoa abnormalities. Evaluation of live spermatozoa (viability) has provisions, namely by looking at every Spermatozoa that do not absorb color means it is alive, while spermatozoa that absorb color means it is dead [13].

The data obtained were analyzed by Analysis of Variance (ANOVA) using SPSS and Duncan's Multiple Range Test [14].

3. RESULT AND DISCUSSION

3.1. Macroscopic Attributes

Sperm volume of ALOBRA was obtained varies between 0.3 - 0.7 ml which was very dependent on the individual response of the rooster during ejaculation. However, the total volume of chicken sperm was still in the normal range about 0.5 ml [8]. The pH of sperm before storage has a pH of 7-9. According to [15] the pH of the rooster sperm, the range is 8.5-9. After storage, the pH of spermatozoa decreased. This is due to the breakdown of fructose which causes the formation of lactic acid. The higher the lactic acid contained in the sperm increased the acidity (pH) of the sperm [16]. The results of this study indicated that the macroscopic quality of ALOBRA used in this study was in normal conditions.

3.2. Sperm Concentration

The concentration obtained in this study was the average sperm concentration when added with NaCl diluent was 641.33 x 109. The spermatozoa concentration in ALOBRA chickens based on the table above showed a significant difference ($P<0.05$).

The addition of propolis, honey, and royal jelly was able to maintain the concentration of spermatozoa for up to 24 hours of storage and specifically for the treatment of honey (P2) was able to maintain a sperm concentration of up to 36 hours of 109.67 x 107. This was because propolis and royal jelly contained carbohydrates as a provider of energy substrates for the needs of spermatozoa in the storage process, as well as in honey there is fructose, which has the same function as carbohydrates. Since long ago it has been very well understood in maintaining the sperm viability and motility that fructose and glucose are a source of main energy for spermatozoa [17]. Carbohydrates serve as a provider of energy substrates for the metabolic needs of spermatozoa in the storage process [18]. In the process of diluting spermatozoa, honey was able to maintain ALOBRA sperm concentration during preservation compared to propolis and royal jelly. It was due to honey, which contains fructose as an energy source while propolis and royal jelly contain only glucose. It was well known that during glycolysis (breakdown), fructolitic activity is better than glycolysis because fructose is a seminal basic sugar [19].

Table 1. Macroscopic Characteristic of Sperm on ALOBRA Rooster diluted by NaCl, Propolis, Honey and Royal Jelly

Treatment		Parameter			
Factor (P)	Factor (A)	Volume (ml)	pH	Colour	Smell
P0	A0	0.75	8	Milky White	Specific smell
	A1	0.70	8	Milky White	Specific smell
	A2	0.40	7	Gray	Specific smell
	A3	0.38	7	Greenish Blue	Specific smell
	A4	0.28	7	Greenish Blue	Specific smell
P1	A0	0.35	8	Light Brown	Specific smell
	A1	0.31	7	Dark Brown	Specific smell
	A2	0.19	7	Dark Brown	Specific smell + Propolis
	A3	0.07	7	Cream	Specific smell + Propolis
	A4	0.02	7	Brown	Specific smell + Propolis
P2	A0	0.60	7	Light Brown	Specific smell
	A1	0.55	7	Light Brown	Specific smell
	A2	0.31	7	Light Brown	Specific smell + Honey
	A3	0.25	6	Light Brown	Specific smell + Honey
	A4	0.12	6	Yellow White	Specific smell + Honey
P3	A0	0.30	7	Milky White	Specific smell
	A1	0.20	7	White	Specific smell
	A2	0.15	6	White	Specific smell
	A3	0.09	6	White	Specific smell
	A4	0.03	6	White	Specific smell

P0: Sperm diluted by NaCl, P1: Sperm diluted by propolis, P2: Sperm diluted by honey, P3: Sperm diluted by royal jelly .

Table 2. Sperm Concentration of ALOBRA stored with different diluent and storage times (x10^7/ml)

Factor A (Time)	Factor P (diluent)				Total	Mean
	Control	Propolis	Honey	Royal Jelly		
0	641.33^{d}	358.67^{abcd}	623.00^{cd}	313.00^{abcd}	1936.00	484.00
12	515.33^{bcd}	208.33^{abcd}	236.00^{abcd}	309.00^{abcd}	1268.66	317.17
24	293.00^{abcd}	139.67^{abc}	109.00^{ab}	103.67^{ab}	645.34	161.34
36	0.00^{a}	0.00^{a}	109.67^{ab}	0.00^{a}	109.67	27.42
48	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00	0.00

Table 3. Sperm Concentration of ALOBRA stored with different diluent and storage times (x10^7/ml)

Factor A (Time)	Factor P (diluent)				Total (A)	Mean
	Control	Propolis	Honey	Royal Jelly		
0	86.00^{g}	79.00^{g}	73.00^{g}	69.00^{fg}	307.00	76.75
12	42.00^{cde}	51.00^{ef}	71.00^{g}	69.00^{fg}	233.00	58.25
24	27.00^{bc}	32.00^{bcd}	50.00^{de}	23.00^{b}	132.00	33.00
36	0.00^{a}	0.00^{a}	16.00^{ab}	0.00^{a}	16.00	4.00
48	0.00^{a}	0.00^{a}	0.00^{a}	0.00a	0.00	0.00

a, b, c: superscript which different on the same column shows the significant difference (P<0,01).

3.3 Sperm Viability

The result showed that a highly significant difference in each treatment (P<0.01) on the percentage of live sperm was observed. The second treatment (P2) on honey was able to maintain sperm life up to above 40% within 24 hours of storage. The honey treatment contained fructose as the main energy of source raw material for the metabolism of spermatozoa. Propolis and royal jelly treatment had carbohydrates that were not better than fructose in honey and NaCl treatment as a control diluent did not have an energy source. However, all the diluents used were able to maintain sperm viability for up to 24 hours at room temperature. ALOBRA chicken sperm mixed with honey can be stored longer up to 36 hours with a percentage of viability that is still suitable for use in artificial insemination.

The percentage of spermatozoa viability was also influenced by storage time. The percentage of spermatozoa viability decreased with increasing storage time due to the availability of energy and food sources for sperm decrease during storage time. Spermatozoa nutrition will be reduced due to the use of energy for mechanical and chemical activities. Spermatozoa metabolism reduced nutrient reserves and electrolyte fluid imbalance which results in damage to spermatozoa cell membranes [19]. The impact of damage on sperm has reduced the function of the membrane, which initially had semipermeable properties. It was no longer able to select the entry and exit of substances so that when the color test was carried out (eosin-negrosin) it entered the plasma. The percentage of live spermatozoa depends on the more spermatozoa that absorb the eosin-negrosin dye solution; the more spermatozoa die [20].

3.3. Sperm Abnormalities

It was observed that no significant difference in each treatment (P>0.05) on sperm abnormalities. Chicken spermatozoa had a fairly high abnormality value and a good abnormality limit of not more than 20%. Spermatozoa morphological abnormalities below 20% were still considered normal. [21]. It was observed that the lowest and highest spermatozoa abnormalities were found in the same treatment, namely honey treatment (P2) of 22% in 0 hours of storage and the highest of 100% in 24 hours of storage. Spermatozoa were not suitable for IB, but the fertility value did not decrease in honey treatment (P2) in 0 hours of storage. Another opinion was that semen that can be used for artificial insemination sperm abnormalities should not be more than 15% and if the spermatozoa abnormalities were more than 25% it will reduce fertility [22]. The honey treatment contains antioxidants obtained from vitamins C and E to maintain the normal metabolism during storage.

Table 4. Viability Sperm (%) of ALOBRA stored with different diluent and storage time

Factor A (Time)	Factor P (diluent)				Total (A)	Mean
	Control	Propolis	Honey	Royal Jelly		
0	86.00^{g}	79.00^{g}	73.00^{g}	69.00^{fg}	307.00	76.75
12	42.00^{cde}	51.00^{ef}	71.00^{g}	69.00^{fg}	233.00	58.25
24	27.00^{bc}	32.00^{bcd}	50.00^{de}	23.00^{b}	132.00	33.00
36	0.00^{a}	0.00^{a}	16.00^{ab}	0.00^{a}	16.00	4.00
48	0.00^{a}	0.00^{a}	0.00^{a}	0.00a	0.00	0.00

a, b, c: superscript which different on the same column shows the significant difference (P<0,01).

Table 5. Sperm abnormalities (%) ALOBRA stored with different diluent and storage time

Factor A (Time)	Factor P (diluent)				Total (A)	Average
	Control	Propolis	Honey	Royal Jelly		
0	36.00	30.00	22.00	32.00	120.00	30.00
12	56.00	63.00	36.00	80.00	235.00	58.75
24	97.00	71.00	37.00	85.00	196.00	72.50
36	0.00	0.00	99.00	0.00	99.00	24.75
48	0.00	0.00	0.00	0.00	0.00	0.00
Total (P)	189.00	164.00	194.00	197.00		
Average	37.80	32.80	38.80	39.40		

a, b, c: superscript which different on the same column shows the significant difference (P<0,01).

Table 6. Sperm motility (%) of ALOBRA stored with different diluent and storage time

Factor A (Time)	Factor P (diluent)				Total (A)	Average
	Control	Propolis	Honey	Royal Jelly		
0	72.00^{c}	57.00^{b}	74.00^{c}	68.00^{c}	271.00	67.75
12	59.00^{b}	0.00^{a}	0.00^{a}	0.00^{a}	59.00	14.75
24	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00	0.00
36	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00	0.00
48	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00	0.00

a, b, c: superscript which different on the same column shows the significant difference (P<0,01).

Acceleration of metabolism increased the percentage of abnormalities in spermatozoa. The acceleration of metabolism was influenced by free radicals that were formed due to the contact of cement with outside air which contains a lot of oxygen during the processing of fresh cement into liquid cement or dilution [23]. Propolis and royal jelly treatments contained antioxidants so that both treatments were also able to maintain the percentage of spermatozoa abnormalities to be better than NaCl at 0 hours and 24 hours of storage. In addition, spermatozoa abnormalities were influenced by genetics and the environment during storage. Storage time may result in abnormalities. The longer the storage time, the higher the percentage of abnormalities caused by cold stress (temperature) and osmotic imbalance due to ongoing metabolic processes in the storage process [24].

3.4. Sperm motility

Spermatozoa motility was one measure of the ability of sperm to fertilize the ovum in the fertilization process. There was a very significant difference in each treatment (P<0.01) on the motility of individual spermatozoa of ALOBRA chickens.

Progressive motile percentage showed that the spermatozoa that have been added to the treatment decreased with the length of storage time at room temperature (25-29^{0}C). The results of that research indicate that the honey treatment (P2) has a fairly high motility of 74% followed by P0, P3, and P1 with

motility of 72%, 68%, and 57% respectively, although in a fairly short storage period for 0 hours. This research also showed an interesting difference, namely being able to provide a longer shelf life at room temperature, which can maintain individual motility for up to 12 hours using 59% NaCl. Compared to a previous study [25] stated that at room temperature storage and using NaCl diluent, it could only maintain 90 minutes with 55% individual motility of Kedu chicken spermatozoa.

Fructose and carbohydrates will be converted into energy in the metabolism of spermatozoa and it does not happen immediately but must go through a process of adaptation. The process of adaptation of spermatozoa to the concentration diluents can result in impaired membrane permeability, decrease cell metabolic activity, damage cells, and decrease the motility of individual spermatozoa [26]. Therefore, damage to the cell plasma membrane will result in disruption of the energy supply and ultimately reduce the motility of spermatozoa. The low motility will eventually lead to a shorter fertile period and spermatozoa fertility.

4. CONCLUSION

Sperm diluent agent that uses honey diluent was able to maintain sperm concentration, viability, and motility values of F1 rooster (ALOBRA) up to 36 hours of storage. In another hand, propolis and royal jelly were only able to maintain the concentration, viability, and motility of ALOBRA sperm up to 24 hours of storage. Honey became the best diluent to extend the storage time of ALOBRA sperm at room temperature until 36 hours. In conclusion, honey contains a better source of energy for sperm to survive longer than other diluent materials and it is very useful in regulating the timing of artificial insemination in hybrid chickens.

AUTHORS' CONTRIBUTIONS

Reza, MA collected sperm of chicken in the field. Abdullah, AN and Koeswara, H carried out a microscopic analysis of sperm. Usman, Y supported the statistical analysis of research results. All authors read and approved the final article.

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