COMPARISON ON THE QUALITY OF LOCAL VILLAGE COCKEREL SPERM USING FOUR DIFFERENT DILUENTS AT ROOM TEMPERATURE

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DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF AGRICULTURAL SCIENCE WITH HONOURS

LIVESTOCK PRODUCTION PROGRAMME
FACULTY OF SUSTAINABLE AGRICULTURE
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LOO HUA BIN
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ABSTRACT

A study was carried out at the Faculty of Sustainable Agriculture (FSA), Sandakan Campus, Universiti Malaysia Sabah (UMS) from August until November 2015. The purpose of this study was to evaluate the coconut water (CW) as natural diluents against chemical diluents for cockerel semen preservation at room temperature (25°C). Four out of 30 local village cockerels were selected for semen collection. Semen collection was repeated twice a week in pooled semen for three replicates of the fresh pooled semen samples with a minimum of 85% individual forward motility. Fresh semen quality was observed based on semen volume, colour and consistency, concentration, mass motility (MMOT) and individual forward motility. Mean volume of the pooled semen was 0.24±0.01 mL, mean colour and consistency of pooled semen was 4.67±0.58 and mean concentration of fresh pooled semen was 1.37±0.02x10⁹ sperm/mL. Moreover, mean MMOT and individual forward motility of fresh pooled semen were 5.67±0.58 and 90.0±0% respectively. Pooled semen samples with more than 85% of individual forward motility were diluted with four different semen diluents, which were Ringer’s solution, TRIS solution, old coconut water (CW) and young coconut water (CW). Pooled semen volume of 20 µL was diluted with 680 µL of the four different diluents in four 2 mL eppendorf tubes separately. This experiment was conducted in the laboratory over a 12 hour intervals after semen collection. Data were taken every hour from four eppendorf tubes for individual forward motility, percentage of live sperm, percentage of head, neck and tail abnormalities and total sperm abnormalities. Ringer’s solution showed the best result in comparison to the other three semen cockerel diluents. It does not showed any significant different (p>0.05) but it showed the highest individual forward motility (46.67±25.17%) in comparison with old CW (6.67±5.77%) at the 12th hour of observation. Ringer’s solution (16.67±1.53%) showed there were significant differences (p<0.01) for live sperm percentage in comparison with old CW (9.33±2.08%) at the same hour of observation. Both of the solutions showed an unstable trend of sperm abnormalities percentage over a 12 hour of intervals. However, TRIS solution and young CW showed a poorer effect on individual forward motility and live sperm percentage where the sperm only survived up to 9th and 5th hour respectively. It was concluded that old CW was comparable to Ringer’s solution and it can be an alternative source for cockerel semen diluents as natural diluents as it is cheaper and readily available all year round in Malaysia and other tropical country.

Keywords : Old coconut water, Ringer’s solution, local village cockerel, cockerel semen diluent
PERBANDINGAN KUALITI AIR MANI AYAM KAMPUNG TEMPATAN DI BAWAH BILIK SUHU DENGAN MENGGUNAKAN EMPAT SPERMA LARUTAN YANG BERBEZA

ABSTRAK

Satu kajian telah dijalankan di Fakulti Pertanian Lestari (FSA), Kampus Sandakan, Universiti Malaysia Sabah (UMS) daripada Ogos hingga November 2015. Tujuan kajian ini adalah untuk mengkaji keberkesanan penggunaan sperma larutan kimia berbanding sperma larutan semula jadi di dalam bilik suhu bagi sperma ayam kampung tempatan. Empat daripada 30 ekor ayam kampung telah dipilih untuk koleksi air mani dan semen dalam kumpulan telah digunakan dalam kajian ini. Eksperimen telah mengulang dua kali seminggu sehingga mendapat tiga kali replikasi sampel yang menunjukkan motiliti individu daripada sampel segar air mani sperma lebih daripada atau sama 85%. Sampel segar air mani sperma telah dikaji berdasarkan jumlah, warna dan konsistensi, kepekatan, motiliti massa dan motiliti individu. Purata bagi jumlah air mani kumpulan adalah 0.24±0.01 mL, purata bagi warna dan konsistensi ialah 4.67±0.58 dan purata kepekatan air mani kumpulan adalah 1.37±0.02 x 10^9 sperma/mL. Tambahannya, purata motiliti massa air mani kumpulan ialah 5.67±0.58 dan motiliti individu ke hadapan daripada sampel segar ialah 90±0%. Motiliti individu ke hadapan lebih daripada atau sama 85% daripada air mani kumpulan daripada sampel segar telah digunakan untuk analisis lanjutan. Jumlah 20μL air mani segar telah dilarut dengan jumlah 680 μL dalam empat buah 2 mL tiub eppendorf yang berasingan dengan empat jenis larutan yang berbeza, iaitu larutan Ringer, larutan TRIS, air kelapa tua dan air kelapa muda. Eksperimen telah dijalankan selama 12 jam berturutan dan data telah dikumpulkan setiap jam sehingga jam ke-12 selepas koleksi air mani untuk mengkaji motiliti individu, peratusan sperma hidup dan peratusan kecacatan sperma dari segi kepala, leher, ekor sperma berserta dengan jumlah kecacatan sperma. Larutan Ringer telah menunjukkan keputusan paling baik berbanding dengan ketiga larutan yang lain. Pada pemerhatian jam ke-12, tiada perbezaan signifikasi (p>0.05) antara larutan Ringer (46.67±25.17%) berbanding dengan air kelapa tua (6.67±5.77%) dari segi motiliti individu. Namun begitu, larutan Ringer menunjukkan tren motiliti individu yang tinggi berbanding dengan air kelapa tua. Pada pemerhatian jam ke-12, terdapat perbezaan signifikasi (p<0.01) antara larutan Ringer (16.67±1.53%) berbanding dengan air kelapa tua dari segi peratusan sperma hidup (9.33±2.08%). Kedua-dua larutan air mani ini telah menunjukkan tren kecacatan sperma tidak stabil sepanjang eksperimen dijalankan; manakala larutan TRIS dan air kelapa muda menunjukkan kesan negatif pada motiliti individu dan sperma hidup. Sperma di dalam larutan TRIS hidup sehingga jam pemerhatian ke-9 manakala sperma di dalam air kelapa muda hidup sehingga jam pemerhatian ke-5. Secara kesimulannya, air kelapa tua boleh bertanding dengan larutan Ringer dan air kelapa tua boleh dijadikan sumber alternatif bagi larutan air mani semula jadi terhadap ayam kampung disebabkan oleh harga yang murah dan boleh didapatkan sepanjang tahun terutamanya di Malaysia dan negara tropikal yang lain.

Kata kunci: Air kelapa tua, larutan Ringer, ayam kampung, larutan air mani ayam kampung
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<td>Degree Celsius</td>
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<tr>
<td>&lt;</td>
<td>less than</td>
</tr>
<tr>
<td>≥</td>
<td>more than or equal</td>
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<td>µL</td>
<td>microlitres</td>
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<td>%</td>
<td>Percentage</td>
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<td>Artificial Insemination</td>
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<td>N-Bis (2-hydroxyethyl)-2-Aminoethane Sulfonic Acid</td>
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<td>BSA</td>
<td>Bovine Serum Albumin</td>
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<td>BTS</td>
<td>Beltsville Thawing Solution</td>
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<td>Ca</td>
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<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
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<td>DOC</td>
<td>Day Old Chick</td>
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<td>DVS</td>
<td>Department of Veterinary Services</td>
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<td><em>et al.</em></td>
<td>Latin: <em>et alia</em> (others)</td>
</tr>
<tr>
<td>Fe</td>
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<td>FSA</td>
<td>Faculty of Sustainable Agriculture</td>
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<td>g</td>
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<td>ITS</td>
<td>Insulin-Transferrin-Selenium</td>
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<td>N-methylacetamide</td>
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<tr>
<td>Met</td>
<td>Methionine</td>
</tr>
<tr>
<td>mL</td>
<td>millilitres</td>
</tr>
<tr>
<td>mm</td>
<td>millimetre</td>
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<td>MMOT</td>
<td>Mass motility</td>
</tr>
<tr>
<td>mOsmol kg⁻¹</td>
<td>Osmolarity of mole per kilogram of water</td>
</tr>
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<td>MVC</td>
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<td>Na</td>
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<td>NaCl</td>
<td>Sodium Chloride</td>
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<tr>
<td>NaHCO₃</td>
<td>Sodium bicarbonate</td>
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<tr>
<td>ND</td>
<td>Newcastle Disease</td>
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<td>P</td>
<td>Phosphorous</td>
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<td>pH</td>
<td>Negative logarithm of the hydrogen ion concentration</td>
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<tr>
<td>®</td>
<td>Registered</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>RT</td>
<td>Room Temperature</td>
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<td>Statistical Analysis System</td>
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<td>SD</td>
<td>Standard Deviation</td>
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<td>TES</td>
<td>N-Tris (hydroxymethyl) methyl-2-Aminoethane Sulfonic Acid</td>
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<td>TRIS</td>
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<td>Thr</td>
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<td>UMS</td>
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</tr>
<tr>
<td>USA</td>
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<td>v/v</td>
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CHAPTER 1
INTRODUCTION

1.1 Background of Study

Poultry production industry plays major roles in the world as it provide food sources for human consumption which has then solved the problems of food security. Product of poultry such as egg and meat contains high protein sources which help in growth and repair of body cell, tissues and bones. Together with no special cultural and religious prohibition, demand towards poultry products increased with increase in human population. It hence contributes to the rises economy of the country. According to Department of Veterinary Services (DVS, 2014), production of poultry industry especially poultry meat production is increased slowly, which was 1.49 million tons in 2014 (Domestic production was 1.437 million tons and importation from other country contributes up to only 0.054 million tons). Meanwhile consumption of poultry products in 2014 was 1.46 million tons. Based on this evidence, Malaysia has reached self-sufficient in poultry meat production as production is higher than consumption or demand.

Commercial broiler production industry had slowly replaced by village chicken in this new era of globalization and modernization. This happened due to human are started looking forward healthy lifestyle and this starts from diets. Changes can make from the diets by consuming more organic food and product, such as village chicken. Village chicken can be said is a high quality meat especially from the aspect of nutritive value and taste which has met the purchasing requirements of consumer. They are reared and grow mostly by farmers at rural areas under zero commercial medicine or drugs system such as antibiotics, hormones and other chemicals substance to avoid residue being leave in the body of chicken even after they were slaughtered. Village chicken are said to be well adapted under harsh environment but productivity are low compared to commercial broiler. Under this circumstance, applicable reproductive...
technology such as artificial insemination (AI) is needed to apply and implemented for genetic advancement and improvement of production for village chicken.

The main constraint of village chicken production is lack of good breeding stock. By introducing AI technique in village chicken, production quality and quantity of village chicken will then increased through the improvement in genetic gain basis. Effort should be mainly focused on optimization on management of cockerel semen storage (Al-Daraji, 2012). Current methods of effective semen storage need to be improved and developed to a better level as it is only efficient up only certain period of time of approximately 12 hours (Thurston, 1995). Quality and quantity of semen are preserved and stored under well managed condition through using of suitable semen diluents and it can be used for long period of time. Natural semen diluents such as coconut water served to increase the volume of semen as semen of cockerel are highly concentrated with low volume. Ejaculated semen needed to be evaluated based on several aspects in order to match the ideal outcome of AI technique.

1.2 Justification

Demand of consumer towards village chicken are increasing with increasing in human population especially when they moving forwards on healthy and organic lifestyle. Demand is higher than production village chicken in Malaysia due to poor husbandry and management system. Development and emphasizing on it are lacking too. Moreover, lack of basic knowledge on the reproductive technology such as AI as well as biochemical mechanisms of semen storage are major obstacles.

Improvement on the genetic basis is needed for a better production of next generation to meet high demand of consumer towards village chicken. Thus, Ringer's solution is chemical diluents which commonly used in semen storage of cockerel. TRIS solution is also example of chemical diluents which used on semen storage of other species of livestock such as goat and yet it can test on storage of cockerel semen. The least common yet economical and readily available natural diluents in Malaysia such as old and young ON are chosen in this study to increase volume and to extend the survivability of cockerel semen under room temperature (RT).
1.3 Objective

Objective of this study is:

1. To evaluate the coconut water (CW) as natural diluents against chemical diluents for cockerel semen preservation at room temperature.

1.4 Hypothesis

Ho: There is no significant difference between coconut water (CW) as natural diluents against chemical diluents for cockerel semen preservation at room temperature.

Ha: There is significant difference between coconut water (CW) as natural diluents against chemical diluents for cockerel semen preservation at room temperature.
CHAPTER 2
LITERATURE REVIEW

2.1  Poultry and Kampong Chicken Production in Malaysia

Poultry species includes fowl, turkey, guinea fowl, duck, geese, quail, pigeons, pheasant, partridges and ostrich or emu. Poultry are live species of animals reared in a range of captivity for special purposes such as breeding, reproduction and production for human consumption. Chicken, duck and quails are example of poultry which are popular under Malaysian context and chicken is still the most popular poultry species consumed by Malaysia due to its high nutritive value especially in protein content, no cultural and religious norm and prohibition (Al-Nasser et al., 2007).

Red jungle fowl species or *Gallus gallus* are ancestors for commercial chicken (Romanov and Weigend, 2001; Hillel *et al.*, 2003; Vaisanen *et al.*, 2005; Al-Nasser *et al.*, 2007). Chicken was evolved for the purpose of cockfighting competition and exhibition in older days (Crawford, 1990; Al-Nasser *et al.*, 2007). Commercial chicken available today were selected based on physical characteristics, such as body size and plumage colour. Crossbreed between Red Jungle Fowl (*Gallus gallus spadiceous*) and mixed exotic domestic breed have produced local village chicken (Petersen *et al.*, 1991). Through evolutionary and artificial selection forces, chicken with superior characteristics were selected prior for commercial production purposes and left out with unselected *Gallus gallus* as local village chicken nowadays.

Village chicken is reared on traditional based with minimal resource input, especially cost. They were kept under backyard system with simple housing materials and mostly fed with kitchen leftover meal. They were fed with supplemented feed such as local grain which was low cost, easily and readily available (Aini, 1990). Moreover, they were characterized by slow maturity rate, small size and low egg production in year round. They are well adapted to any harsh environment with highly resistance to disease or illnesses due to environmental and evolutionary forces. Malaysian who was
consuming village chicken made selection based on their present superior phenotypic characteristics such as body weight (BW) as it is the one of the major sole criterion. Thus, consumer normally purchased local village chicken with higher percentage of carcass dressed yield of edible portion to BW as it was denoting a better equivalent of economic background (Azlina and Engku, 2011).

2.2 Anatomy and Reproductive Physiology of cockerel

Testes or gonad served as primary sexual organ under context of reproduction system in cockerel. It is located in the internal body cavity of animal which differs from some other mammals (Malik et al., 2013). It was located at the back of cockerel near the top of kidneys. They are elliptical and show light yellow in colour. It possessed function which includes producing testosterone, the primary male sex hormones and sperm. Sperm will be only produced when sexual maturity is attained in cockerel, which is about five months of age.

According to Etches (1996), the size of two testes may differ from each and another, by right testis usually are lighter than left testis at 0.5-3.0g and this has correlation with amount of semen produced, such as the larger the size of testes, the greater the sperm production (Senger, 2003). Production of sperm or spermatogenesis will happened at 41°C in cockerel which was slightly different from mammal which they produced sperm when scrotal temperature in the range of 24-26°C (Tuncer et al., 2006). Reproductive tract of cockerel consists of a duct system with a paired of epididymis and vas deferens. Diameter of vas deferens will increased before copulation and this was to allow semen stored in the bulbous region and semen was then released from vas deferens when there is sexual stimulation (Perry, 1960).

2.3 Semen Collection Technique

Semen was collected using semen collection technique prior to improve the genetic basis of poultry through AI. Before carrying out real semen collection process, cockerel needs to be trained in order to produce semen that can be used in AI. Cockerel are trained to familiar with methods carried out by AI technician and at the same time letting AI technician familiar with semen collection in cockerel for smooth launching of the whole semen collection process. Whole semen collection process needed to take care to maximize the quantity and quality of semen collected later on as contamination from the collecting equipment, foreign material such as blood and faeces might caused error or inaccurate of the result (Lukaszewicz, 2002).
Abdominal massage technique of semen collection served as traditional yet effective methods used as described by Burrows and Quinns (1937) and it was practicing until today. Cockerel which managed under semi-intensively is taken gently from the cage with minimal stress and the abdomen region of cockerel was massaged and followed by the tail is being pushed forward over the back of cockerel. Consequence of this massage methods leads to erection of copulatory organ followed by semen produced and secreted from ejaculatory ducts. In order to avoid any unwanted wastage of semen, it must manipulate immediately as reflex massage is difficult to elicit together with ejaculation once the first excitement was missed. Some of the factors such as quality, quantity and cleanliness of ejaculated sperm may affect by pressure exerted on the ejaculatory ducts (Maule, 1962). Sperm and semen having a strong correlation in achieved successful reproduction. Factor associated in degeneration of the seminiferous tubule from external or environmental factors such as nutrient deficiency in diets, either partially or fully degenerated may affect spermatogenesis which then leading to poor sperm production resulted from semen collected (Anderson, 2001).

2.4 Semen Extender

Poultry semen was characterized by viscose and highly concentrated. Semen extender or diluents was used to increase the volume of semen especially those animal produce low volume of semen per ejaculation in order to inseminate more female animal such as hen per semen ejaculation in cockerel. It is one of the important procedures for approaching successful AI depending to what extent semen can be preserved and stored to maintain the fertility (Sukumarannair et al., 2004).

It can be stored in terms of short and long term condition. Short term storage of semen (less than three days) can be stored as liquid while long term storage of semen (more than three days) can be stored in frozen condition depending on favour of poultry breeder. Semen stored and preserved under extenders shows good quality sperm. Moreover, the viability and fertilizing ability being affected under diluted condition compared to undiluted semen (Siudzin’ska and Łukaszewicz, 2008). There is a significant decrease in the percentage of dead sperm under dilution condition (Clarke et al., 1984; Dumpala, et al., 2006a). Health risk caused in AI also reduced under this circumstance (Reed, 1982; Sukumarannair et al., 2004).
2.4.1 Types of Semen Extender

a) General and Specific Types for Avian Species

There are some of the aspects, which were identical to seminal plasma was provided to spermatozoa. Semen extenders were prepared based on these aspects. The aspects includes, an appropriate energy source, pH and osmolarity levels which were identical to seminal plasma. One of the most important constituent of avian seminal plasma is glutamic acid. During preparation of avian semen extender, it has become a references aspect to refer for (Lake and McIndoe, 1959; Siudzin’ska and Łukaszewicz, 2008). Availability of special buffered salt solutions can be used as cockerel semen extender whereby variable of components can be included depending on the types of semen extender.

Adrohep, Beltsville thawing solution (BTS), Cornell University extender (CUE) are example of conventional extender which were being used in the old days, while egg yolk, honey and CW were local well-known semen extender (Pitso, 2009). They can be work alone or cooperate with other semen extender in preserving semen. Currently, palm wine plus "Nche" (Saccoglotis gabonensis) was discovered as one of the semen extenders too (Umesiobi, 2004). Spermatozoa should be protected by extender from cold shock injury especially during cooling. Egg yolk or skimmed milk can be added to meet this requirement. Buffers were needed to add to maintain and stabilize the pH in range of 7.0-7.4 as well as prevent any changes in the semen extender. Examples of buffers include sodium citrate, sodium phosphate and TRIS. Moreover, egg albumin, skimmed milk, CW, gentamicin sulphate or antibiotics such as streptomycin can be added into extender as additives (Siudzin’ska and Łukaszewicz, 2008).

2.4.2 Application of Coconut Water (CW) (Cocos nucifera) as Semen Extender

a) Used in Other Species

CW was often used as semen extender and named as CW extender in different type of livestock species. CW was widely distribute and readily available all year round. Coconut water solution (CWS) was added together with some supplementation such as pyruvate, glutamine, hypoxanthine, insulin-transferrin-selenium (ITS) and bovine serum albumin (BSA) were used for preservation of preantral follicle (Silva et al., 2000) and semen (Cardoso et al., 2002). In order to improve the frozen-thawed sperm
viability and avoid quality of frozen-thawed semen of swine being degraded, osmolarity of CW has to decrease until the level of osmolarity are lesser than 200 mOsmol kg\(^{-1}\) before adding into freezing diluents (Luzardo \textit{et al.}, 2010) and hence CW can act as one of the natural semen diluents in poultry species.

**b) Used in Avian**

CW or endosperm of \textit{Cocos nucifera} is an alternative solution as semen extender like does saline due to its special sterility and weak acid based characteristics. It is the liquid found in the central cavity of coconut which represents 25% weight of the fruits. There is approximately 95.5% was water basis, carbohydrates and fat content with 4% and 0.1% respectively. Moreover, content of iron (Fe), calcium (Ca) and phosphorous (P) are 0.5%, 0.02% and 0.01% respectively. There were presence of amino acid, vitamin C, vitamin B complex and mineral salt too (Vigliar \textit{et al.}, 2006). The presence of high concentration of sodium (Na) ion and potassium (K) ion in CW with the presence of arginine (Arg) and lysine (Lys) which were then serves as crucial factors for reversible suppression motility and survivability of sperm. With the addition of honey bee, it has improved the sperm motility of agouti (\textit{Dasiprocta aguti}) (Silva \textit{et al.}, 2011). There is no research has been done using CW as semen diluents on avian species. Thus, CW can be serving as another new source of semen diluents especially on avian species.

**2.5 Semen Preservation**

Semen preservation either short or long term has made artificial insemination (AI) more possible to carry out and achieve at higher successful rate. Semen was usually preserved under room temperature, chilled temperature and through methods of cryopreservation. Methods of semen preservation were depended on decision of breeder. Even after the death of cockerel, its sperm still can be used to inseminate hen through extending the storage period of sperm under appropriate storing temperature for example, frozen state of temperature (Latif \textit{et al.}, 2005).

**2.5.1 General Technique for Semen Preservation**

**a) Room Temperature (RT)**

Room temperature (RT) is one of the techniques for poultry semen preservation. Sperm motility in domestic chicken (\textit{Gallus gallus}) can be affected when temperature are increased to range of 30-40 °C. Moreover, quality changes in the medium are
highly correlated to interaction of several factors such as ion composition level, temperature and pH (Bonato et al., 2012). Under this circumstance, the sperm motility will be at normal range at 40 °C under alkalization of pH with inclusion of calcium (Ca) (Ashizawa and Wishart, 1987; Ashizawa et al., 1994). Thus, the interaction effect of storage medium pH, storage temperature needed to be taken into consideration to maintain sperm viability and activity in vitro.

b) Cryopreservation

Dispersion and conservation of germplasm of livestock can be achieved through cryopreservation. It is one of the assisted reproductive technologies besides AI (Bonato et al., 2012), where Chicken was became the first livestock species where it carried out on (Lake, 1986; Donoghue and Wishart, 2000; Blesbois and Brillard, 2007). It was called as sperm bank and one of the most efficient methods for conserving genetic basis of poultry species. It is a method of preserving the genetic resource using species specific cryoprotectants under ex situ management for endangered species of livestock (Ehling et al., 2012).

2.5.2 Preservation of Cockerel Semen

Cockerel sperm motility and fertilizing ability shows a deceasing trend within one hour after collection especially it is stored in vitro (Dumpala et al., 2006a). Special and unique physiological characteristics have caused cockerel semen shown up a different reacting trend under normal freezing condition (Ehling et al., 2012). Application of an appropriate amount of glycerol (an example of cryoprotectants) of less than 0.7% with volume/volume percent (v/v) = 0.1 M has proposed a good surviving environment for rooster semen which preserved under post-thaw condition (Ehling et al., 2012) and it may lead to successful result of AI (Long and Kulkarni, 2004). Other examples of cryoprotectants used include dimethyl sulfoxide (DMSO), dimethylformamide (DMF), dimethylacetamide (DMA) or N-methylacetamide (MA). Successful rate were also depending on several factors such as concentration of cryoprotectant, equilibration temperature and time, freezing rate, method and post-thaw treatment. Under this circumstance, DMSO shows the most toxic and least effective result compare to the others (Nik Iylia, 2014); while DMA, DMF and MA shows effective result in freezing semen of cockerel with respect to the factors involved (Ehling et al., 2012).
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