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

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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 $1.37\pm0.02x10^9$ 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 eppendoft tubes separately. This experiment was conducted in the laboratory over a 12 hour intervals after semen collection. Data were taken every hour from four eppendoft 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 bawah suhu bilik terhadap 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 $1.37\pm0.02x10^9$ sperma/ mL. Tambahan pula, purata motiliti massa air mani kumpulan ialah 5.67±0.58 dan motilit 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 bagi analysis lanjutan. Jumlah 20µL air mani segar telah dilarut dengan jumlah 680 µL di dalam empat buah 2 mL tiub eppendoft 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 bersertaan 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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°C	Degree Celsius
<	less than
≥	more than or equal
µL	microlitres
%	Percentage
AI	Artificial Insemination
ANOVA	Analysis of Variance
Arg	Arginine
BES	N-Bis (2-hydroxyethyl)-2-Aminoethane Sulfonic Acid
BSA	Bovine Serum Albumin
BTS	Beltsville Thawing Solution
BW	Body Weight
Ca	Calcium
$CaCl_2 \cdot 2H_2O$	Calcium chloride dihydrate
$C_6H_{12}O_6$	Glucose
$C_6H_8O_7$	Citric acid
$C_6H_{12}O_6$	Fructose
CRD	Completely Randomized Design
CT	Chilled Temperature
CUE	Cornell University Extender
CW	Coconut Water
CWS	Coconut Water Solution
DMA	Dimethylacetamide
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DOC	Day Old Chick
DVS	Department of Veterinary Services
et al.	Latin: *et alia* (others)
Fe	Iron
FSA	Faculty of Sustainable Agriculture
g	Gram
ITS	Insulin-Transferrin-Selenium
K	Potassium
KCl	Potassium Chloride
LSD	Least Significant Differences
Lys	Lysine
MA	N-methylacetamide
Met	Methionine
mL	millilitres
mm	millimetre
MMOT	Mass motility
mOsmol kg^{-1}	Osmolarity of mole per kilogram of water
MVC	Malaysia Village Chickens
Na	Sodium
NaCl	Sodium Chloride
$NaHCO_3$	Sodium bicarbonate
ND	Newcastle Disease
P	Phosphorous
pH	Negative logarithm of the hydrogen ion concentration
®	Registered

RT	Room Temperature
SAS	Statistical Analysis System
SD	Standard Deviation
TES	N-Tris (hydroxymethyl) methyl-2-Aminoethane Sulfonic Acid
TRIS	Tris (Hydroxymethyl) Aminomethane
Thr	Threonine
UMS	Universiti Malaysia Sabah
USA	United State of America
v/v	volume per volume

LIST OF FORMULAE

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 CW 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 *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 *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 *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 (*Dasiprocta aguti*) (Silva *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 *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 (*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).

REFERENCES

Abdul Rashid, B., Julie Marzlinda, M. R. and Zurain, R. 2014. Efficacy of Coconut Water as Extender for Savanna Goat Bucks. *Proceedings of the 1st ASEAN Regional Conferences on Animal Production and 32nd MSAP Annual Conferences*, 4-6 June 2014, Kuching, Sarawak. 47-48

Abutu, J. A. 2011. *Effects of Diluents, Age of Semen and Insemination Doses on Viability and Fertility of Turkey Semen Preserved at Ambient Temperature.* Bachelor of Animal Science Dissertation. Univerisity of Nigeria, Nsukka

Aini, I. 1990. Indigenous Chicken Population in South-East Asia. *World's Poultry Science Journal* **46**: 51-57

Al-Daraji, H. J. 2012. Effect of Diluents Supplementation with Tomato Juice on Semen Quality and Storage Ability of Roosters' Semen during Liquid Storage. *International Journal of Biology, Pharmacy and Allied Science* **1(7)**: 918-926

Alkan, S., Baran, A. Ozdas, B. O. and Evecen, M. 2002. Morphological Defects in Turkey Semen. *Journal Veterinary Animal Science of Turkey* **26**: 1087-1092

Al-Nasser, A., Al-Khalaifa, H, Al-Saffar, A., Khalil, F., Al-Bahouh, M., Ragheb, G., Al-Haddad, A. and Mashaly, M. 2007. Overview of Chicken Taxonomy and Domestication. *World's Poultry Science Journal* **63**: 285-300

Ameen, S. A., Opayemi, O. S., Ajayi, J. A. and Adediwura, M. A. 2014. Evaluation of Semen Quality of Five Different Cockerel Bred Used in Poultry Industry in Nigeria. *Journal of Environmental Issues and Agriculture in Developing Countries* **6 (1)**: 30-36

Anderson, J. 2001. The Semen of Animals and Its Use for Artificial Insemination. Greenworld Publishers India

Ashizawa, K. and Sano, R. 1990. Effects of Temperature on the Immobilization and the Initiation of Motility of Spermatozoa in the Male Reproductive Tract of the Domestic Fowl, *Gallus domesticus*. Comparative of Biochemistry and Physiology **96(2)**: 297-301

Ashizawa, K., Wishart, G. J., Nakao, H., Okino, Y., Tsuzuki, Y., 1994. Inhibition of Temperature-Dependent Immobilization of Fowl Spermatozoa at Body Temperature by an Increased Intracellular pH. *Journal of Reproduction and Fertility* **101**: 593-598

Ashizawa, K., Wishart, G. J., 1987. Resolution of the Sperm Motility Stimulating Principle of Fowl Seminal Plasma into Ca^{2+} and an Unidentified Low Molecular Weight Factor. *Journal of Reproduction and Fertility* **81**: 495-499

Azlina, A. I. A. and Engku, A. E. A. 2011. Dressed Yield and Edible Parts of Crossbred Village (Kampung) Chicken as Affected by Restrictions in Feed. *Malaysian Society of Animal Production* **14**: 57-60

Bajpai, P. K. and Brown, K. E. 1964. The Effect of Different Temperatures on the Metabolic Activity, Morphology and Fertilizing Capacity of Turkey Semen. *Poultry Science* **43**: 1501-1508

Bakst, M. R. and Cecil, H. C. 1997. Determination of Sperm Concentration I, II and III. In: Bakst, M. R. and Cecil, H. C. (eds) *Techniques for Semen Evaluation, Semen Storage and Fertility Determination*. Poultry Science Association, Illinois. 6-22

Bakst, M. R. 1980. Fertilizing Capacity and Morphology of Fowl and Turkey Spermatozoa in Hypotonic Extender. *Journal of Reproduction and Fertility* **60**: 121-127

Blanco, J. M., Wildt, D. E., Hofle, U., Voelker, W. and Donoghue, A. M. 2009. Implementing Artificial Insemination as an Effective Tool for *Ex Situ* Conservation of Endangered Avian Species. *Theriogenology* **71**: 200-213

Blesbois, E. 2006. Advances in avian semen cryopreservation [CD-ROM]. In: Romboli I, Flock DK, Franchini A (eds) 12th European Poultry Conference, 10-14 September 2006, Verona, Italy: Abstracts and Proceedings. Ithaca, NY: World's Poultry Science Association

Blesbois, E. 2007. Current Status in Avian Semen Cryopreservation. *World's Poultry Science Journal* **63**: 213-222

Blesbois, E. and Brillard, J. P. 2007. Specific Features of In Vivo and In Vitro Sperm Storage in Birds. *Animal* **1**: 1472-1481

Blesbois, E., Grasseau, I., Seigneurin, F. Mignon, G. S., Saint, J. M. and Mialon, R. M. 2008. Predictors of Success of Semen Cryopreservation in Chickens. *Theriogenology* **69 (2)**: 252-261

Blom, E. 1981. Physiology and Pathology of Reproduction. *Medycyna Weterynaryjna* **4**: 239-242

Bogdonoff, P. D. and Schaffner, C. S. 1954. The Effect of pH on In Vitro Survival, Metabolic Activity and Fertilizing Capacity of Chicken Semen. *Poultry Science* **33**: 665-669

Bonato, M., Cornwallis, C. K., Malecki, I. A., Rybnik, T. P. K. and Cloete, S. W. P. 2012. The Effect of Temperature and pH on the Motility and Viability of Ostrich Sperm. *Animal Reproduction Science* **133**: 123-128

Bottini, L., Castro, C. M., Gambao, F. A, Lopez, M. A. and Lopez, A. A. 2010. Osmolarity of Coconut Water (*Cocos nucifera*) Based Diluents and their Effect over Viability of Frozen Boar Semen. *American Journal of Animal and Veterinary Sciences* **5(3)**: 187-191

Brillard, J. P. 2003. Practical Aspects of Fertility in Poultry. *World's Poultry Science Journal* **59**: 441-446

Brown, H. B. and McCartney, M. G. 1986. Restricted Feeding and Reproductive Performance of Individually Caged Broiler Breeder Males. *Poultry Science* **65 (5)**: 850-855

Burrows, W. H. and Quinn, J. P. 1937. The Collection of Spermatozoa from the Domestic Fowl and Turkey. *Poultry Science* **24**: 19-24

Cardoso, R. C. S., Silva, A. R., Uchoa, D. C. and Silva, L. D. M. 2002. Cryopreservation of Canine Semen using Coconut Extender with Egg Yolk and Three Different Glycerol Concentrations. *Theriogenology* **59**: 743-751

Christensen, V. 1995. Diluent, Dilution and Storage of Poultry Semen for Six Hours. In: Bakst M, Wishart G, editors. *Proceedings of the First International Symposium on Artificial Insemination of Poultry.* Illinois. Savoy. 90-106

Clarke, R. N., Bakst, M. R. and Ottinger, M. A. 1984. Morphological Changes in Chicken and Turkey Spermatozoa Incubated under Various Conditions. *Poultry Science* **63**: 801-805

Cole, H. H. and Cupps, P. T. 1977. Reproduction in Domestic Animals. 3rd ed. New York. Academic Press

Crawford, R. D. 1990. Origin and History of Poultry Species. Poultry Genetic Resources: Evolution, Diversity and Conservation. In: Crawford, R. D. (Ed.). *Poultry Breeding and Genetics*. Elsevier, Amsterdam. 1-59

Das, S. K. 2002. Effects of Feeding on Semen Production in Native Cock in Bangladesh. *Journal of Biological Science* **2**: 810-811

Donoghue, A. M. and Wishart, G. J. 2000. Storage of Poultry Semen. *Animal Reproduction Science* **62**: 213-232

Dumpala, P. R., Parker, H. M., and McDaniel, C. D. 2006a. The Effect of Semen Storage Temperature and Diluent Type on the Sperm Quality Index of Broiler Breeder Semen. *International Journal of Poultry Science* **5(9)**: 863-845

Dumpala, P. R. Parker, H. M. and McDaniel, C. D. 2006b. The Sperm Quality Index from Fresh Semen Predicts Chicken Semen Quality after Storage. *International Journal of Poultry Science* **5(9)**: 850-855

DVS. 2014. Malaysia: Consumption of Livestock Products, 2005-2014. http://www.dvs.gov.my/documents/10157/5b1cbd30-54f3-4bf3-824c-0aa328f5775f. Accessed on 7 July 2015. Verified on 8 July 2015

Ehling, C., Taylor, U. Baulain, U., Weigend, S., Henning, M. and Rath, R. 2012 Cryopreservation of Semen from Genetic Resource Chicken Lines. *Agriculture and Forestry Research* **3 (62)**: 151-158

Etches, R. J. 1996. *Reproduction in Poultry.* 1st edition. CAB International, Cambridge, United Kingdom

Galal, A. 2007. Predicting Semen Attributes of Naked Neck and Normally Feathered Male Chickens from Live Performance Traits. *International Journal of Poultry Science* **1**: 36-42

Gordon, I. 2005. Reproductive Technologies in Farm Animals. CABI Publishing United Kingdom

Graham, J. K. 2001. Assessment of Sperm Quality: A Flow Cytometric Approach. *Animal Reproduction Science* **68(3-4)**: 239-247

Hafez, B. and Hafez, E. S. E., 2000. Reproduction in Farm Animals. 7th ed. New York. Lippincott Williams and Wilkins, United State of America

Hammerstedt, R. H. and Shultz, F. T. 1995. Long-Duration Semen Holding: Description, Consequences and Preparations for Optimum Use. *Turkeys* **43**: 22-25

Helmenstine, A. M. 2014. Ringer's Solution Recipe. http://chemistry.about.com/od/labrecipes/a/Ringer-S-Solution-Recipe.htm. Access on 1 July 2015. Verified on 2 July 2015

Hillel, J., Groenen, M. A., Tixier,B. M. Korol., A. B., David, L., Kirzhner, V. M., Burke, T., Baarre, D. A., Crooijmans, R. P., Elo, L., Feldman, M. W., Freidlin, P. J., Maki, T. A., Oortwijn, M., Thomson, P., Vignal, A., Wimmers, K and Weigend, S. 2003. Biodiversity of 52 Chicken Populations Assessed by Microsatellite Typing of DNA Pools. *Genetics Selection Evolution* **35**: 533-557

Holstein, A. F., Roosen, R. E. C. and Schirren, C. 1988. Illustrated Pathology of Human Spermatogenesis. *Grosse Verlag*

Howarth, B. J. 1983. Fertilizing Ability of Cock Spermatozoa from the Testis, Epididymis, and Vas Deferens following Intramagmal Insemination. *Biology Reproduction* **28**: 586-590

Hu, S., Zhang, X. G., Han, C., Wei, S. Y., Xie, D. Q., Du, R. R. and Hu, J. H. 2015. Effects of Three Different Diluents on Quality of Boar Semen Stored at 17°C. *Journal of Northeast Agricultural University* **22(2)**: 36-46

Hudson, B. P. and Wilson, J. L. 2003. Effects of Dietary Menhaden Oil on Fertility and Sperm Quality of Broiler Breeder Males. *Journal of Applied Poultry Research* **12**: 341-347

Kelso, K. A., Cerolini, S., Noble, R. C., Sparks, N. H. C. and Speake, B. K. 1996. Lipid and Antioxidant Changes in Semen of Broiler Fowl from 25 to 60 Weeks of Age. *Journal of Reproduction and Fertility* **106(2)**: 201-206

Kotlowska, M., Glogowski, J., Dietrich, G. J., Faruga, A., Jankowski, J. and Ciereszko, A. 2005. Biochemical Characteristics and Sperm Production of Turkey Semen in Relation to Strain and Age of the Males. *Poultry Science* **84(11)**: 1763-1768

Kuster, C. E., Singer, R. S. and Althouse, G. C. 2004. Determining Sample Size for the Morphological Assessment of Sperm. *Theriogenology* **61(4)**: 691-703

Lake, P. E. 1966. A Cytochemical Examination of Spermatozoa of the Domestic Fowl. *Research in Veterinary Science* **7**: 121-127

Lake, P. E. 1986. The History and Future of the Cryopreservation of Avian Germ Plasma. *Poultry Science* **65**: 1-15

Lake, P. E. and McIndoe, W. M. 1959. The Glutamic Acid and Creatine Content of Cock Seminal Plasma. *Journal of Biochemistry* **71**: 303-306

Lake, P. E. and Ravie, O. 1984. An Explanation of Cryoprotective Compounds for Fowl Spermatozoa. *Poultry Science* **61**: 145-150

Lake, P. E. and Stewart, J. M. 1978. Artificial Insemination in Poultry. Bulletin 213 of the Ministry of Agriculture Fisheries and Food, HMSO, London

Lake, P. E. and W. M. McIndoe. 1959. The Glutamic Acid and Creatine Content of Cock Seminal Plasma. *Biochemical Journal* **71**: 303-306

Latif, A., Ijaz, A., Aleem, M. and Mahmud, A. 2005. Effect of Osmotic Pressure and pH on the Short Term Storage and Fertility of Broiler Breeder Sperm. *Pakistan Veterinary Journal* **25(4)**: 179-182

Long, J. A., Bongalhardo, D. C., Pelaez, J., Saxena, S., Settar, P., Sullivan, N. P. O. and Fulton, J. E. 2010. Rooster Semen Cryopreservation: Effect of Pedigree Line and Male Age on Post-Thaw Sperm Function. *Poultry Science* **89(5)**: 966-973

Long, J. A., Kulkarni, G. 2004. An Effective Method for Improving the Fertility of Glycerol-Exposed Poultry Semen. *Journal of Poultry Science* **83**: 1594-1601

Lukaszewicz, E. 2002. An Effective Method for Freezing white Italian Gander Semen. *Theriogenology* **58**: 19-27

Lukaszewicz, E., Jersey, A., Partyka, A. and Siudzinska, A. 2008. Efficacy of Evaluation of Rooster Sperm Morphology using Different Staining Methods. *Research in Veterinary Science* **85**: 583-588

Luzardo, B., Castro, M. C., Gamboa, F. A., Lopez, M. A. and Lopez, A. Y. R. A. 2010. Osmolarity of Coconut Water (*Cocos nucifera*) Based Diluents and their Effect Over Viability of Frozen Boar Semen. *American Journal of Animal and Veterinary Sciences* **5(3)**: 187-191

Makhafola, M. B., Umesiobi, D. O., Mphaphathi, M. L., Masenya, M. B. and Nedambale, T. L. 2012. Characterization of Sperm Cell Motility Rate of Southern African Indigenous Cockerel Semen following Analysis by Sperm Class Analyser. *Journal of Animal Science Advances* **2(4)**: 416-424

Malik, A., Haron, A. W., Yusoff, R., Nesa, M., Bukar, M. and Kasim, A. 2013. Evaluation of the Ejaculate Quality of the Red Jungle Fowl, Domestic Chicken, and Bantam Chicken in Malaysia. *Turkish Journal of Veterinary and Animal Sciences* **37**: 564-568

Maria, B. L., Fernando, C. C., Militza, A. G., Ricardo, A. P. and Jose, H. C. 2013. Effect of Addition of Coconut Water (*Cocos nucifera*) to the Freezing Media on Post-Thaw Viability of Boar Sperm. *Tropical Animal Health Production* **45**: 101-106

Maule, J. P. (Ed.). 1962. *The Semen of Animals and Artificial Insemination.* Commonwealth Agricultural Bureaux, Farnham Royal, Bucks

Moce, E. and Graham, J. K. 2006. Cholesterol-Loaded Cyclodextrins added to Fresh Bull Ejaculates Improve Sperm Cryosurvival. *Journal of Animal Science* **84**: 826-833

Mosenene, T. M. B. 2009. *Characterization and cryopreservation of semen of four South African chicken breeds. Degree of Master of Science Thesis.* University of the Free State, Bloemfontein

Nik Iylia, H. 2014. *The Survivability of Village Chicken Sperm using Two Levels of Glycerol in Chilled Ringer's Diluents.* Bachelor of Science Dissertation. Universiti Sultan Zainal Abidin

Nunes, J. F. 1993. Coconut Water (*Cocos nucifera*) in Full Nature Added with Cytokinin as Goat Semen Diluents. *Review of Science* **3(3)**: 273

Nwachukwu, E. N., Ibes. N. and Amadi, C.U. 2006. Effects of Genotype and Frequency of Semen Collection on Semen Characteristics of Local Chicken Cocks. *Journal Animal Veterinary Advance* **5**: 562-565

Omeje, S. S. I., Marire, B. N. 1990. Evaluation of the Semen Characteristics of Adult Cocks of Different Genetic Backgrounds. *Theriogenology* **3**: 1111-1118

Parker, J. E. and Arscott, G. H. 1963. Energy Intake and Fertility of Male Chickens. *Journal of Nutrition* **82**: 183-187

Perry, E. J. 1960. The Artificial Insemination of Farm Animals. Rutgers University Press, New Jersey

Peters, S. O., Shoyebo, O. D., Ilori, B. M., Ozoje, M. O., Ikeobi, C. O. N. and Adebambo, O. A. 2008. Semen Quality Traits of Seven Strains of Chickens Raised in the Humid Tropics. *International Journal of Poultry Science* **7(10)**: 949-953

Petersen, J. B., Guzman, J. M. R. D. and Wu, M. C. 1991. Catalogue of the Native Poultry of Southeast Asia. Food and Fertilizer Technology Centre for Asian Pacific Region, Taiwan. Taiwan Livestock Research Institute. Taiwan

Pitso, T. 2009. Improvements in the Viability and Fertilizing Integrity of Boar Spermatozoa using the "Umqombothi" *Sorghum Bicolour* Semen Extenders. Degree of Master Science Thesis. Central University of Technology of the Free State

Pornjit, S., Thevin, V. and Banyat, L. 2013. Effects of Environmental Factor, Age and Breeds on Semen Characteristics in Thai Indigenous Chicken: A One-Year Study. *Thai Journal of Veterinary Medicine* **43(3)**: 347-352

Quinn, J. P. and Burrows, W. H. 1936. Artificial Insemination in Fowls. *Journal of Heredity* **27**: 31-37

Reed, H. C. B. 1982. Commercial Requirements for an Effective Fresh Semen Diluent. In: *Proceedings of the 2nd International Conference on Boar Semen Preservation.* 19-22 August 1990. Beltsville, Maryland. 255-270

Renema, R. A., Rustad, M. E. and Robinson, F. E. 2007. Implications of Changes to Commercial Broiler and Broiler Breeder Body Weight Targets Over the Past 30 Years. *World's Poultry Science Journal* **63**: 457-472

Romanov, M. N. and Weigend, S. 2001. Analysis of Genetic Relationships between Various Populations of Domestic and Jungle Fowl using Microsatellite Markers. *Poultry Science* **80**: 1057-1063

Sansone, G., Nastri, M. J. R. and Fabbrocini, A. 2000. Storage of Buffalo (*Bubalus bubalis*) Semen. *Animal Reproduction Sciences* **62**: 55-76

Santiago, M. J., Castano, C., Toledano, D. A., Coloma, M. A., Lopez, S. A., Prieto, M. T. and Campo, J. L. 2011. Influence of Season on the Freezability of Free-Range Poultry Semen. *Reproduction in Domestic Animal* **47(4)**: 578-583

Santoso, U., Kubo, K., Ota, T., Tadokoro, T. and Maekawa, A. 1996. Nutrient Composition of Kopyor Coconuts (*Cocos nucifera* L.). *Food Chemistry* **57**: 299-304

Senger, P. L. 2003. *Pathways to Pregnancy and Parturition.* 2nd edition. Pullman, Washington, United State of America

Sexton, T. 1988. Comparison of Commercial Diluents for Holding Turkey Semen 24 Hours at 48°C. *Poultry Science* **67**:131-134

Sexton, T. J. 1976. Studies on the Dilution of Turkey Semen. *British Poultry Science* **17**: 179-186

Silva, J. R. V., Lucci, C. M., Carvalho, F. C. A., Báo, S. N., Costa, S. H. F., Santos, R. R. and Figueiredo, J. R. 2000. Effect of Coconut Water and Braun-Collins Solutions at Different Temperatures and Incubation Times on the Morphology of Goat Preantral Follicles Preserved *In Situ. Theriogenology* **54**: 809-822

Silva, M. A., Peixoto, G. C. X, Santos, E. A. A., Castelo, T. S., Oliveira, M. F. and Silva, A. R. 2011. Recovery and Cyopreservation of Epididymal Sperm from Agouti (*Dasiprocta aguti*) using Powdered Coconut Water (ACP-109c) and Tris Extenders. *Theriogenology* **76**:1084-1089

Siudzin´ska, A. and Łukaszewicz, E. 2008. Effect of Semen Extenders and Storage Time on Sperm Morphology of Four Chicken Breeds. *Journal of Applied Poultry Research* **17**: 101-108

Sukumarannair, S. A., Alejandro, L., John, D., Robert B. M., Luke, M. 2004. A Retrospective Study on the Preserving Capacity of a Commercial Boar Semen Extender. *Theriogenology* **62 (3)**: 425-436

Tabatabaei, S, Batavani, R. A. and Talebi, A. R. 2009. Comparison of Semen Quality in Indigenous and Ross Broiler Breeder Roosters. *Journal of Animal and Veterinary Advances* **8(1)**: 90-93

Thurston, R. 1995. Storage of Poultry Semen above Freezing for 24-48 Hours. In: Bakst, M. R. and Wishart, G. J. (Eds.). *Proceedings of the First International Symposium on Artificial Insemination of Poultry.* Illinois, Savoy. 107-122

Thurston, R. J., Rogoff, M. S., Scott, T. R. and Korn, N. 1993. The Effects of Perfluorochemical Diluent Additives on the Fertilizing Capacity of Turkey Semen. *Poultry Science* **72**: 598-602

Tuncer, P. B., Kinet, H., Ozdogan, N. and Demiral, O. 2006. Evaluation of Some Spermatological Characteristics in Denizli Cocks. *Journal of Faculty Veterinary Medicine University* **3**: 37-42

Umesiobi, D. O. 2004. Functional Integrity of Boar Spermatozoa and Sow Fertility using Raphia (*Raphia hookeri*) Palmwine plus 'Nche' (*Saccoglotis gabonensis*) Urban Extender. *Journal of Applied Animal Research* **26**: 13-16

Vaisanen, J., Hakansson, J. and Jensen, P. 2005. Social Interaction in Red Junglefowl (*Gallus gallus*) and White Leghorn Layers in Stable Groups and after Re-grouping. *British Poultry Science* **46(2)**: 156-168

Vasconcelos, R., Ferreira, N. J., de Mello, S. C. C. Maciel, C. J. M., Alencarde, M. A. and Alencarde, A. A. 2009. Avaliação morfológica de espermatozoides caprinos diluídos e congelados em meio à base de água de coco em pó (acp-101) ou tris, corados por eosinanigrosina e azul de bromofenol (Abstract in English). *Ciência Animal Brasileira* **10**: 862-869

Vigliar, R, Sdepanian, V. L. and Fagundes, N. U. 2006. Biochemical Profile of Coconut Water from Coconut Palms Planted in an Inland Region. *Journal of Pediatria* **82**: 308-312

Watson, P. F. 1990. Artificial Insemination and Preservation of Semen. In: *Lamming G. Marshall's Physiology of Reproduction*. 4th edition. Churchill Livingstone, Edinburgh, England. 747-869

Wishart, G. J. and Wilson, Y. I. 1999. Temperature Dependent Inhibition of Motility in Spermatozoa from Different Avian Species. *Animal Reproduction Science* **57 (3-4)**: 229-235

Zahraddeen, D., Butswat, I. S. R., Kalla, D. J. U., Sir, S. M. and Bukar, M. T. 2005. Effect of Frequency of Ejaculation on Semen Characteristics in Two Breeds of Turkeys (*Meleagris gallopavo*) Raised in a Tropical Environment. *International Journal of Poultry Science* **4**: 217-221