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Research Article

The effect of semen extender and storage time on the quality of spermatozoa collected from the excurrent duct of Philippine local chicken

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Abstract: The study was conducted primarily to determine the effect of AU and Lake's Low Temperature (LLT) extenders and to determine the effect of extended semen at 0 hour, 24 hours, 48 hours and 72 hours storage times. Twelve roosters were used for the collection of spermatozoa from the excurrent duct (epididymis and vas deferens) of Philippine local chickens. During the time of semen storage, a decrease in the number of motile, live, morphologically normal spermatozoa and an increase in dead and abnormal spermatozoa were observed. The LLT extender was found to be a more suitable extender because the number of live spermatozoa did not decrease as much as in semen diluted with the AU extenders. In addition, the best time storage determined was between 0 hour to 24 hours on both extenders, however, results showed that microscopic evaluation at 24 hours to 48 hours in LLT extender had comparable semen quality.

INTRODUCTION

Biodiversity and genetic conservation are of broad interest all over the world. Although, conservation focuses on endangered animals, gene conservation in livestock animals is equally important. Since domestic chicken is the most important domestic avian species for the industrial production of meat and eggs [1] intensive breeding and homogeneity of environment conditions were done for maximum production, however the genetic diversity of domestic species had decreased rapidly [2].

Cryopreservation of sperm is the only efficient method for *ex-situ* management of avian genetic materials [3]. Despite the cryopreservation method, the overall fertility rates of poultry frozen sperms remain highly variable and not reliable enough for commercial production or preservation of genetic stocks [4]. In addition, the fertility rates of cryopreserved poultry semen are dramatically lower than any of the domestic mammalian species [5].

In order to maintain the fertilizing ability of semen for long time, semen extenders with various additives are needed [6]. An appropriate semen extender has to provide an energy source for spermatozoa and maintain pH and osmolarity levels identical to those of seminal plasma, the natural medium for sperm [7].

This research aimed to determine the effect of two different extenders that will prolong the semen quality of Philippine local chicken as well as to determine the storage time that will prolong the motility and viability of the spermatozoa.

MATERIALS AND METHODS

Twelve sexually matured roosters were used for the collection of excurrent ducts by blunt dissection. Quickly, the excurrent ducts were placed in a 1.5 ml eppendorf tube with 400 µl of Phosphate Buffer Saline (PBS). The semen collected was divided into two and was placed in eppendorf tube with 1:1 ratio, volume of semen: volume of extender, both for AU and LLT extenders. The extended semen was evaluated considering the sperm motility, viability and abnormality. Extended semen was stored in the refrigerator at a temperature of 4°C. Evaluation of extended semen was at 0 hour, 24 hour, 48 hour and 72 hours period.

Semen Motility Evaluation

The sperm motility was assessed by placing 10 µl of the semen in a clean slide and was gently covered with a cover slip. The percentage motility of spermatozoa was evaluated according to the motility scoring system [8]. Motility evaluation was done at 0 hour, 24 hour, 48 hour and 72 hour.

Evaluation of Live Sperm

Semen smear was prepared on a microscope slide with the mixture of 10ul semen and 20ul of Eosin-Nigrosin staining solution by Jaskowski. Stained samples were evaluated using an inverted microscope. The number of live and dead spermatozoa was determined. Sperms that are dead absorbed the stain and appeared pink/purple, and those that did not absorb the stain were the live sperms. At least 200 representative samples of spermatozoa were counted in different microscopic fields. Evaluation of live sperms was done at 0 hour, 24 hour, 48 hour and 72 hour. Percent live sperm was determined using the formula shown below.

$$\% \text{ Live sperm} = \frac{\text{live spermatozoa}}{\text{total spermatozoa counted}} \text{ x } 100$$

Evaluation of Sperm Morphology

In the examination of sperm morphology, 10 µl sperm samples were mixed with 20 µl Eosin-Nigrosin Solution for more than one minute. The diluted sperm samples were smeared on a microscope slide, dried for 10 minutes and were evaluated using an inverted microscope. The shape of the head, neck or mid piece and the tail were examined. At least 200 sperm cells were counted in different microscopic fields. Semen abnormality of the extended spermatozoa was evaluated at 0 hour, 24 hour, 48 hour and 72 hour. Percent abnormal sperm was calculated using the formula shown below.

$$\% \, Abnormality = \frac{abnormal \; sperm}{total \; spermatozoa \; counted} \; x \; 100$$

Statistical analysis

This study used t-test for independent samples (SPSS version 27) to compare AU and LLT extender at different microscopic evaluation periods.

RESULTS AND DISCUSSIONS

Sperm Motility

Table 1 presents the percent motility of extended semen using LLT and AU at different storage time. Extended semen at 0 hour using AU and LLT have percent motility of 64.58 ± 5.42 and 65.83 ± 5.57, respectively. Semen motility decreased over time in both semen extenders although extended semen that used LLT showed higher percent motility in 24 hour, 48 hours and 72 hours evaluation with 65.83%, 51.11%, 38.13% and 26.70 %, respectively, as compared to extended semen that used AU , 64.58%, 40.56%, 26.56% and 17.75%, respectively.

Percent Sperm Viability

Percent live sperms in both treatments were observed highest in 0 hour with 81.97% and 73.99% for LLT extended semen and AU extended semen, respectively. The viability of AU and LLT extenders at 0 hour has the highest percent viability. Semen extended with LLT extender was observed consistently higher compared to AU extender with percent live sperms of 81.97%, 74.34%,70.49% and 65.21% at 0 hour, 24 hour, 48 hour and 72 hour storage time, respectively, and 73.99%, 70.32%, 59.11% and 55.24%, respectively.

Percent Sperm Morphology

The same trend was observed as to the abnormalities of the extended semen using AU and LLT semen extenders. At 0 hour, extended semen using both extenders had the lowest observed abnormalities. It was observed that the percentage of spermatozoa in LLT extender was lower than the percentage of abnormal spermatozoa in AU extender in all storage times of observation. It proved further that LLT semen extender is better than AU semen extender.

Table 1. Effect of AU and LLT extenders on the motility of spermatozoa at different storage times

Extender	Sperm Motility,%			
	0 Hour	24 Hours	48 Hours	72 Hours
AU Extender	64.58 ± 5.42	40.56 ± 22.00	26.56 ± 17.27	17.75 ± 16.63
LLT Extender	65.83 ± 5.57	51.11 ± 21.33	38.13 ± 22.19	26.70 ± 17.16

Table 1. Effect of AU and LLT extenders on the livability of spermatozoa (mean ± SD) at different storage times

Extender	Percent Live Sperm			
	0 Hour	24 Hours	48 Hours	72 Hours
AU Extender	73.99 ± 10.37	70.32 ± 10.37	59.11 ± 10.26	55.24 ± 11.34
LLT Extender	81.97 ± 6.16	74.34 ± 8.73	70.49 ± 8.75	65.21 ± 12.07

Table 2. Effect of AU and LLT extenders on the sperm abnormality (mean ± SEM) at different storage times

Extender	Percent Abnormal Sperm			
	0 Hour	24 Hours	48 Hours	72 Hours
AU Extender	7.76 ± 4.63	9.72 ± 4.55	13.76 ± 5.31	15.08 ± 5.71
LLT Extender	4.65 ± 1.96	7.70 ± 2.95	8.42 ± 1.01	10.26 ± 4.40

CONCLUSIONS

LLT extender was found to be a better extender over AU in terms of the parameters tested: motility, viability and sperm normality. Over time, LLT extended semen registered higher percentage motility, viability and higher percent normal sperms when evaluated after 24, 48 and 72 hours which can be attributed to the LLT extender composing of monosodium glutamate which is highly correlated to the survivability of spermatozoa .

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REFERENCES

1. Rocha JR.J, Mahecha G, Dornas R, Kuana S, and Martins N. Epididymallithiasis in Gallus gallusdomesticus. Brazilian Journal of Poultry Science.doi:10.1590/S1516-635X2009000100004. May, 2009.
2. Tisdell C. Socioeconomic causes of loss of animal genetic biodiversity: analysis and assessment. Ecol Econ 2003, 45: 365–376.
3. Ehling C,Taylor U, Baulain U, Weigend S, Henning M and Rath D. Cryopreservation of semen from genetic resource chicken lines. Agriculture and Forestry Research 2012, 3:151-158.
4. Long AJ .Avian Semen Cryopreservation: What Are the Biological Challenges? Poultry Science 2006, 85, 232–236.
5. Gersilov V. Influence of various cryoprotectant on the sperm mobility of Muscovy semen before and after cryopreservation. Agricultural Science and Technology 2010, 2:57-60.
6. Kubicova E, Riha V, Pivco J, Apolen D and Makarevich A. Effect of different semen extenders and additives to insemination doses on ewe´s pregnancy rate. Slovak J. Anim. Sci, 2010, 3: 118-122. Retrieved from ISSN 1337-9984.
7. Siudzinska A and Lukaszewicz E. (2008). Effect of Semen Extenders and Storage Time on Sperm Morphology of Four Chicken Breeds. Journal of Applied Poultry Research. doi:10.3382/japr.200, 00048. 101-108
8. Mamuad FV, Venturina EV and Saito H. Collection Processing and handling buffalo semen. Water buffaloes and beef cattle improvement project. A joint JICA assisted project of the Philippine Carabao Center and the Bureau of Animal Industry, Philippines2004.

About Author

David G. Antalan III, graduated at Central Luzon State University, April 2015 with Bachelor of Science in Biology. He is currently working on the Molecular identification and morphological characterization of Philippine swamp buffalo and different livestock species for efficient management of genetic materials and tissues at the Animal Genetic Resources, Cryobank Unit, Philippine Carabao Center National Headquarters and Gene Pool, Science City of Muñoz, Nueva Ecija, Philippines.