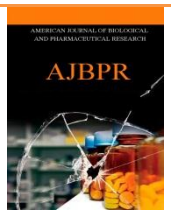
e-ISSN - 2348-2184
Print ISSN - 2348-2176

AMERICAN JOURNAL OF BIOLOGICAL AND PHARMACEUTICAL RESEARCH

Journal homepage: www.mcmed.us/journal/ajbpr



COMPARISON OF THE QUALITY OF EXTENDED PHILIPPINE NATIVE CHICKEN (*Gallus gallus domesticus*) SPERM COLLECTED BY ABDOMINAL MASSAGE METHOD

Heinz Kesniel D. Guieb[1*], Marcos B. Valdez[2] and Flocerfida P. Aquino[3]

[1]Biology Program, Sciences Cluster, University of the Philippines Cebu, Cebu City, Philippines.
[2]Department of Biological Sciences, Institute of Arts and Sciences, Far Eastern University, Manila, Philippines 11000.
[3]Reproductive Biotechnology Laboratory, Philippine Carabao, Center Science City of Muñoz, Nueva Ecija, Philippines 3120.

Article Info

Received 15/11/2015
Revised 26/11/2015
Accepted 12/12/2015

Key words: -
Cryopreservation, semen extender, Cryobank.

ABSTRACT

This study aimed to compare the use of two semen extenders after several hours of storage, i.e. 0h, 6h, 12h, 24h, 36h, 48h and 72h. Two chicken extenders were compared in the study: Lake's Low Temperature (LLT) extender and AU extender. Semen was collected from six male chickens and was mixed with the extenders in a 1:1 ratio. Sperm motility, morphology and concentration were determined. Results showed that AU extender was able to produce viable sperms having a motility of $\geq$ 30% after 24 h of storage at 5^{o}C. On the other hand, LLT extended semen motility dropped to as low as 18% after 6 hours of storage at 5^{o}C. However, both extenders were able to produce an acceptable range of normal sperm morphology ($\geq$ 70%). The work conducted can be used for future studies for the cryopreservation of native chicken sperm and cryobanking purposes in the Philippines.

INTRODUCTION

In the Philippines, the native chicken is important due to the preference of locals because of its distinctive taste and these native breeds are also important since they are more resistant and more adaptable to environmental changes [1]. In developed countries, different native breeds are important because of its traditions and cultural values and the emergence of niche markets for livestock products. In developing countries, the importance of livestock breeds is focused on the food security and economic development [2]. Poultry is one of the most important commodities among the livestock in Southeast Asian countries and these animals are raised either for meat or egg production [3].

Corresponding Author

Heinz Kesniel D. Guieb
Email:- heinzniel12@gmail.com

In 2010, the estimated population of chicken in the Philippines was at 159 million, about 50 percent of the total chicken population was accounted for native or village chicken which was raised in backyard farms [4].

There are a growing percentage of the genetically diverse poultry stocks which were developed by academic researchers, have disappeared or have become endangered in recent years. Southeast Asian countries tend to have a low appreciation of their own local breeds due to the assumption that exotic breeds are better, this led to the native breeds becoming endangered [3]. Furthermore, these species could be affected by natural calamities such as global warming. There is an increasing loss of genetic diversity agriculturally used species, and poultry genetic resources are considered to be the most endangered [5]. Initiatives such as cryopreservation are being undertaken to preserve the native breeds. Cryopreservation is done to preserve genetic lines of different animal species.

The ability to cryopreserve and store germplasm has been valued for the preservation of genetic material, especially for at-risk populations [6]. In the Philippines, the Philippine Carabao Center is spearheading in the cryopreservation of native animal breeds like buffaloes and goats. However, the effort in the cryopreservation of native chicken is still at its very early stage. This initiative was done to characterize the sperm of the native chicken. The researchers in the Philippines, being an archipelagic country, face the challenge of transporting the germplasm from the field to the cryobank facility which is located at the Philippine Carabao Center Headquarters in Science City of Muñoz, Nueva Ecija.

The cryopreservation of the sperm of these breeds would be helpful in retaining and in conserving their genetic material. This research aimed to determine which extender could efficiently prolong the motility of chicken sperm which would provide a baseline data for the cryopreservation of chicken sperm in the Philippines.

MATERIALS AND METHODS

Semen collection

Six sexually matured roosters were used for the extraction of sperm. Abdominal massage method was used in collecting the semen following the method described [7-8]. The rooster was immobilized by placing it between the legs of the handler. The wings were secured and the legs were extended posteriorly. Force was applied to the pelvis of the rooster to stimulate the testis and squeezed the cloaca for the collection of semen into the 1.5 ml tube. The semen was stored at 5^{o}C in a Styfoam box and was brought to the laboratory for quality evaluation. Semen motility, morphology and semen concentration data of the neat semen were checked prior to semen extension and storage at 5^{o}C.

Cooling of Chicken Sperm

After collection, the semen collected was divided into 3 and was placed in 3 separate small tubes and were diluted to a ratio of 1:1 (semen : extender) based in a previously reported method [6]. After addition of the extender, evaluation at 0h was done and cooling at 5^{o}C inside the refrigerator followed immediately. Evaluation of semen quality at different storage hours was done thereafter.

Evaluation of Semen Quality

Semen Motility

The percentage motility of spermatozoa was evaluated using the scoring system [9]. A drop of the semen was placed on a clean glass slides and was observed using an inverted microscope. Semen motility evaluation was done at 0h, 6h, 12h, 24h, 36h, 48h and 72h.

Sperm Concentration

Concentration of the sperm was determined by counting the sperm using a haemocytomer. Only the Neat/fresh semen sample was used for the determination of sperm concentration. Diluting the sperm to a ratio of 1:200 (semen: semen diluting fluid) was done and the semen mixture was counted.

Sperm Morphology

This was determined by mixing the semen with the eosin-nigrosin stain. At least 200 sperm cells were counted in different microscopic fields per semen sample. Semen abnormalities were observed and compared the extended semen using LLT and AU at different storage period.

Statistical Analysis

Data gathered were analyzed using Microsoft Excel 2013® and SPSS ®. One way analysis of variance (ANOVA) and t-test were used to determine the level of significance among the means of the data gathered. The results were presented as mean $\pm$ SD.

RESULTS AND DISCUSSION

Sperm Motility

Motility of the extended semen at different observation hours is presented in Table 1. Motility of AU extended semen at 0h observation was significantly different ($p \leq 0.05$) from 24, 36, 48 and 72h observations with percent motility of 61.67$\pm$ 7.64, 31.67$\pm$ 2.89, 26.67 $\pm$ 2.89, 18.33$\pm$ 11.55 and 13.00$\pm$ 1.73, respectively. Comparing the motility of LLT extended semen, at 0h observation it differed significantly ($p \leq 0.05$) for the rest of the observation period, from 6, 12,24, 36, 48 and 72h with percent motility of 65.00$\pm$ 5.00, 18.33$\pm$ 2.89, 17.67$\pm$ 2.89, 16.67$\pm$ 7.64, 18.33$\pm$ 5.77, 10$\pm$ 0.00 and 12.00 $\pm$ 13.23, respectively. LLT extended semen started to differ significantly ($p \leq 0.05$) from the AU extended semen and the control at 6h. This is where the LLT extended semen decreased dramatically. Assessment of sperm motility is one of the most often used parameters for semen evaluation [10-13]. The range reported for normal cock semen motility was between 40-80% [12-15]. However the sperm is still acceptable between 30-40% though it is not that desirable [2]. Lake's extender appeared to have less beneficial effect on stored semen motility as it declined significantly after being stored for 6h at 5^{o}C.

The slope for each trend line, shown in Figure 1 below, showed the rate of decrease for each group. The rate of decrease for the motility of the sperm between groups was significantly different ($p \leq 0.05$). The highest rate of decrease in sperm motility among the three groups was the Lake's Extender. On the other hand, the Extender AU has the slowest rate of decrease based on the mean of the slope.

Furthermore, it significantly dropped after 6 hours of observation. The life of the sperm proved to be at its most critical at the 12-h mark as shown by the performance of the control group.

Sperm Morphology

The sperm of the chicken differs to that of the mammals. It has a straight head and the tail looks like a mere extension of the head [8]. The poultry sperm is very critical in cryopreservation since it is susceptible to freezing damage due to the morphology of its head [8]. Observed percentage of normal sperm of extended semen is summarized in Table 2. There were no significant differences for each observation. Sperm morphology is one of the most essential qualitative characteristics of poultry semen [16; 13] since it can be used as a parameter in knowing the fertilizing capabilities of the spermatozoa [13; 17]. The percentage abnormal sperms in this study were of acceptable range, all were lower than 30%. Proportions of more than 30% in abnormalities of spermatozoa are not viable for fertilization [2].

Sperm Concentration

The resulting average sperm concentration of all the chicken sperm samples was $3.62x10^9$ /ml. This result was consistent with the studies done [18] with the jungle fowl having a sperm concentration of $3.19\ x10^9$ /ml. Sperm concentration is an important indicator of the viability of the sperm [2;13;19].

Table1. Motility of extended semen using AU and LLT at different observation periods

Observation Time (h)	Control,%	AU Extender, %	LLT Extender, %
0	60.00± 5.00[a]	61.67 ± 7.64[a]	65.00± 5.00[a]
6	50.00± 5.00[a]	36.67 ± 2.89[a]	18.33± 2.89[b]
12	25.67± 2.89[a]	40.00 ± 8.66[b]	17.67± 2.89[a]
24	27.50± 3.54[a]	31.67± 2.89[a]	16.67± 7.64[a]
36	23.33± 2.89[a]	26.67± 2.89[a]	18.33± 5.77[a]
48	13.33± 2.89[a]	18.33±11.55[a]	10.00± 0.00[a]
72	11.67± 2.89[a]	13.00± 1.73[a]	12.00±13.23[a]

Means with different superscripts indicate significance at P≤ 0.05

Table2. Percentage normal spermatozoa of extended semen using AU and LLT at different observation periods

Observation Time (h)	Control,%	AU Extender, %	LLT Extender, %
0	91.33± 1.15	94.67 ± 0.58	95.67± 1.53
6	88.67± 1.53	88.00 ± 2.65	82.67± 3.79
12	83.00± 2.00	83.67 ± 4.04	83.33± 5.13
24	8400 ± 3.00	87.00 ± 3.61	85.00± 3.00
36	81.67± 1.53	79.33± 1.53	76.33± 3.51
48	79.33± 2.08	81.33± 3.06	81.00± 1.73
72	75.33± 1.53	79.33± 1.15	78.33±2.52

Data are presented as Mean ± SD.

Figure1. Sperm motility trend of the control, AU extended, and LLT extended semen.

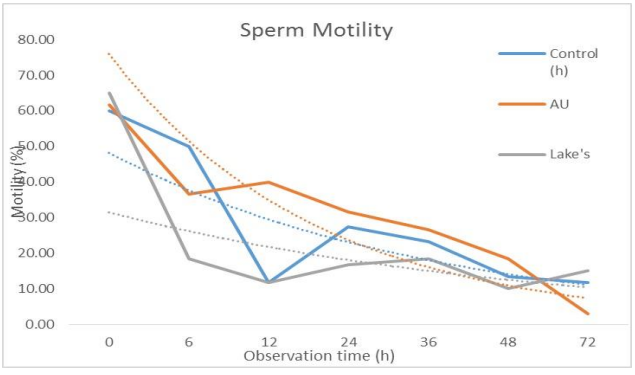
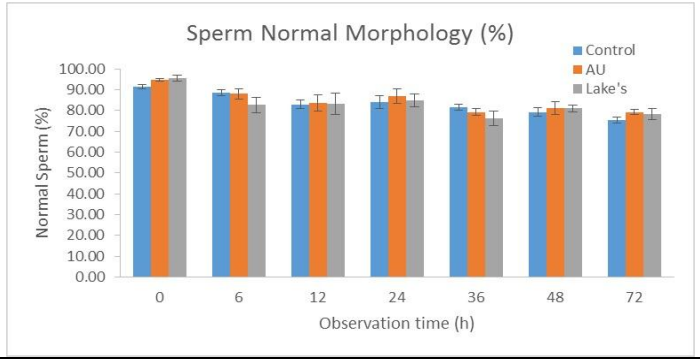


Figure 2. showed the observed percentage of normal sperm of the control, AU extended semen and LLT extended semen. There were no significant differences (P ≤ 0.05) for each of the observations



CONCLUSIONS AND RECOMMENDATIONS

Both of the extenders are efficient in producing morphologically normal sperms, having percent normality rates of more than 70%. The AU extender is recommended to be used as the sperm extender up until the 24 h of storage time. Moreover, it can be utilized for future chicken sperm preservation studies.

It is recommended to use three or more sperm extenders for the determination of the efficacy of the extender, more so in the determination of extender to be used for the cryopreservation of native chicken sperm. The researchers recommend the Extender AU to be used for further studies.

ACKNOWLEDGMENT

The main author is very thankful to Drs. Lerma C. Ocampo and Eufrocina P. Atabay and to all the Reproductive Biotechnology Unit staff for their motivation and support during the conduct of his research.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Salces AJ, Quirog LM, Chatto EI. (2010). Participatory approach in definition of breeding objective traits for Boholano strain of native chicken. *Philippine Journal of Veterinary and Animal Sciences*, 39(2), 165 – 172.
2. FAO. (2012). Cryoconservation of animal genetic resources. FAO Animal Production and Health, Guidelines No. 12. Rome.
3. Gunawan, B. (1990). Endangered breeds of poultry and ducks. FAO Animal Production and Health Paper. 95-106.
4. PCAARRD. (2012). Industry status – chicken.
5. Weigend S, Romanov MN, Rath D. (2004). Methodologies to identify, evaluate and conserve poultry genetic resources. Food and Agriculture Organization of the United Nations, 1-103
6. Long, JA, Kulkarni G. (2004). An effective method for improving the fertility of glycerol-exposed poultry semen. *Poultry Science Association,* 1594 – 1601.
7. Burrows, WH, Quinn JP. (1937). The collection of spermatozoa from the domestic fowl and turkey. *Poult. Sci*, 26, 19–24.
8. Donoghue AM, Wishart GJ. (2000). Storage of poultry semen. *Anim. Reprod. Sci*, 62, 213-232.
9. Mamuad FV, Venturina EV, Saito H. (2004). 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, Philippines.
10. Froman, DP, Feltmann AJ. (1998). Sperm mobility: A quantitative trait of the domestic fowl (Gallus domesticus). *Biol. Reprod*, 58, 379–384.
11. Holsberger, DR, Donoghue AM, Froman D P, et al (1998). Assessment of ejaculate quality and sperm characteristics in turkeys: Sperm mobility phenotype is independent of time. *Poultry Sci*, 77, 1711–1717
12. King, LM, Holsberger DR., Donoghue AM. (2000). Correlation of CASA velocity and linearity parameters with sperm mobility phenotype in turkeys. *J. Androl*, 21, 65–71.
13. Malik A, Haron AW, Yusoff R. et al. (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.
14. Lake, P.E. (1966). A cytochemical examination of spermatozoa of the domestic fowl. *Res. Vet. Sci*, 7, 121–127.
15. Omeje, SSI, Marire, BN. (1990). Evaluation of the semen characteristics of adult cocks of different genetic backgrounds. *Theriogenology*, 3, 1111–1118.
16. Kuster, CE, Singer, RS, Althouse, GC. (2004). Determining sample size for the morphological assessment of sperm. *Theriogenology*, 61, 691–703.
17. Lukaszewicz, E. (1998). Study of diluents of cock's semen storage in the light of laboratory estimation and fertility rate. *Poult. Sci*, 43–59.
18. Orunmuyi M, Akanwa CL, Nwagu BI. (2013). Semen quality characteristics and effect of mating ratio on reproductive performance of hubbard broiler breeders. *Journal of Agricultural Science*, 5(1), 154-159.
19. Birkhead, TR, Martinez, JC, Burke, T., et al. (1999). Sperm motility determines the outcome of sperm competition in the domestic fowl. *Proc. Biol. Sci*, 266, 1759–1764.