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Research Article

Comparison of quality of spermatozoa from the excurrent ducts of Philippine local chicken retrieved by swim-up or mincing methods

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Abstract: *This study sought to compare various parameters of spermatozoa retrieved by swim-up method (SUM) and mincing method (MM) from the excurrent ducts of Philippine local chicken. Spermatozoa were collected aseptically from the excurrent ducts of twelve sexually matured Philippine local chickens by blunt dissection and were subsequently retrieved by mincing and swim-up method. Semen parameters such as motility, viability, sperm concentration and percent abnormality of the samples were determined. Results showed that the motility, viability, concentration and percent abnormality of spermatozoa retrieved by SUM were not significantly different from that of spermatozoa retrieved by MM which indicated that the method used for the retrieval of spermatozoa from the excurrent ducts did not in a way influence the resulting aforementioned semen parameters. It was therefore concluded that both of the retrieval methods used in this study yielded semen samples with comparable semen parameters and that good quality spermatozoa could be collected from the excurrent ducts of Philippine local chicken.*

INTRODUCTION

The development, application and improvement of reproductive technology, modern genetic tools in breeding programs and global logistics, respectively, resulted to a significant increase in animal production and enabled rapid genetic progress in production traits. However increased use of highly productive breeds lead to a decrease in genetic diversity in most species of farm animals [1]. Conservation of genetic resources and of biodiversity is one of the major focuses of many researches nowadays. In situ conservation of genetic resources is the approach prioritized by every conservation program involving animal species [2]. One drawback of the *in situ* type of conservation is its inability to ensure the protection and conservation of target species due to live animals' maintenance complexity [3]. The loss of valuable genes and rapid decrease in biodiversity as a result of smaller number of selected breeds used for breeding has resulted in urgent need to create gene banks and databanks [4,5]. In poultry species, semen cryopreservation is currently the most practical method for long-term storage of genetic material [2]. Complex knowledge of the sperm and its physiology, along with proper diluents and dilution rates, are essential to achieve maximum success [6]. Due to high variability in success rates of semen cryopreservation for the ex situ management of genetic diversity in birds, the need for predictors of semen freezability is underscored [2]. Semen evaluation prior to its further processing is essential and an important prerequisite. The assessment of the basic quality of sperm is indispensable for the purpose of devising methods for the storage of fowl spermatozoa in vitro for long periods of time [7]. The quality of whole fresh semen, which can be measured by classical morphologic, metabolic and mobility tests, may serve as a reliable indicator of the suitability of semen for cryopreservation. Only good quality ejaculates are used for cryopreservation as they are more likely to withstand the cryopreservation and thawing process [2]. Characteristics of good quality semen include: pearly white in color; free of any contamination with cloacal products; volume of greater than 0.3 mL; sperm mobility of greater than 65%; and sperm concentration greater than 1 x 10^9 sperm cells/ml. In a study conducted [2] , it was reported that different in vitro tests of semen quality, with membrane fluidity test being the most accurate are reliable in determining the freezing ability of the gametes and, thus, the success rate of chicken semen cryopreservation. These in vitro tests are essential to the improvement of freezing

methods and for optimizing the management of frozen semen [2,8].

Dorso-abdominal massage is the most frequently used method of semen collection in birds [9]. However, urine contamination, a common and lethal acidifier of fresh semen ejaculates in wild raptors which results in reduced sperm viability and motility, is a problem that may be encountered in this method. This could be circumvented by the immediate and gentle seminal washing [10]. Other contaminants include feces and blood, which tend to reduce or worse, infect the collected semen. Furthermore, collection via abdominal massage requires a trained technician to avoid semen contamination. Post-mortem epididymal sperm retrieval is one way to overcome the problems encountered in collection by abdominal massage and studies on the evaluation of spermatozoa retrieved from the excurrent ducts of Philippine roosters have yet to be done. The present study is aimed to compare various parameters of spermatozoa collected from the excurrent ducts of Philippine local rooster retrieved by swim-up and mincing methods.

MATERIALS AND METHODS

Sample collection

Twelve sexually matured roosters were used for the collection of excurrent ducts which were done as aseptically as possible by blunt dissection. The semen samples were retrieved using the swim-up and mincing methods. The procedure was carried out with the help of a licensed veterinarian.

Swim-up method

Following the method [11], microcentrifuge tubes with PBS were just left to stand for 10-15 minutes. The upper liquid portion was transferred to another conical tube. Microscopic evaluation was conducted to assess the following parameters from the retrieved spermatozoa: concentration, gross motility, individual motility, viability and percent abnormality.

Mincing method

The excurrent ducts were placed in microcentrifuge tubes containing 400µl of PBS and were minced using sterile surgical scissors and were left to stand for 10-15 minutes. The upper liquid portion was transferred to another microcentrifuge tube. Microscopic evaluation was conducted to assess the following parameters of the retrieved spermatozoa: concentration, gross motility, individual motility, viability and percent abnormality.

Microscopic evaluation

Motility

A drop of semen (10 µL) was placed on a clean, pre-warmed (41°C) microscope slide using a micropipette. A clean cover slip was placed gently to avoid air bubble formation. Sperm motility was demonstrated by the progressive wavy movement of sperm cells [12]. The scoring system [13] was used for the evaluation of the movement of sperm to rate the sperm motility.

Sperm viability

Ten microliters of semen sample was mixed with 20ul of eosin-nigrosin solution and a smear was prepared. Sperm viability was done counting at least 200 sperms from the smeared semen sample on the glass slide representing the live and the dead sperms [13]. The dead sperms absorb the stain and appear pink/purple and the sperms that do not absorb the stain are the live sperms.

The number of live sperms was counted and the percentage of live sperms was computed using the formula:

$$Viability\ (\%) = \frac{live\ spermatozoa}{total\ spermatozoa\ counted}\ x\ 100$$

Sperm abnormality

In the examination of sperm morphology, 10 µL sperm sample was mixed with 20 µL eosin-nigrosin solution and smear was prepared. The samples were evaluated using bright field microscopy. The shape of the head, neck or mid piece and the tail were examined. Sperm cells with abnormal morphology were counted and were expressed as mean percentage of sperm head, neck or tail abnormality. At least 200 spermatozoa were counted in different microscopic fields [13]. The percentage of abnormal spermatozoa was computed using the formula:

$$Percent\ abnormality = \frac{abnormal\ sperm}{total\ number\ of\ sperm\ observed} \times 100$$

Sperm concentration

Five microliters of the sample was added to 995 µL of 3% NaCl solution in microcentrifuge tube. Ten microliters of the mixture were then dispensed to each of the chambers of the haemocytometer. The number of spermatozoa in five large squares from the middle square of each chamber was counted. The head of the spermatozoa was considered as the reference in counting. As some spermatozoa transcended the lines at the edge of the squares, only spermatozoa on the top right lines were included in the count. The average of the spermatozoa from the two counting chambers was calculated. The concentration of spermatozoa per milliliter of semen was computed by multiplying the average spermatozoa by 10^7 [12].

Statistical analysis

The results obtained were expressed as mean ± SEM and were analyzed using t-test for two independent samples to determine the differences in semen quality using the statistical software IBM® SPSS Statistics version 21. P values ≤ 0.05 were considered significant.

RESULTS AND DISCUSSIONS

The study on the comparison of quality of spermatozoa retrieved from the excurrent ducts of Philippines local chicken retrieved by swim-up and mincing methods was conducted to evaluate and characterize spermatozoa from the ducts initiating from the epididymis to the ductus deferens, collectively referred to as the excurrent ducts. Two methods, the mincing and swim-up methods, were used to retrieve the

spermatozoa from the isolated excurrent ducts. Parameters such as motility, viability, concentration, and abnormality were determined to assess which of the two retrieval methods could be utilized to collect good quality spermatozoa which are paramount in the development of methods of poultry spermatozoa cryopreservation. Table 1 presents the comparison of motility, viability, concentration and percent abnormality observed in the samples retrieved using swim-up method (SUM) and mincing method (MM).

Sperm Concentration

The table shows that the concentrations of sperm (billion cells per mL) retrieved by swim-up and mincing methods were found to be 2.08 ± 1.1 and 1.93 ± 1.17, respectively. T-test found no significant difference between the concentrations of samples retrieved using swim-up and mincing methods. Normal sperm concentration varies both within and between individuals even in the same species [14], but it is estimated [15] to be normally between of 1.7 to 3.5 billion sperm cells per milliliter for chicken, and the results in this study confirmed to the mentioned figures.

The concentration of samples retrieved from the excurrent ducts, regardless of the retrieval method used, were found to be within the considered normal range in ejaculated samples. Results implied that concentrations of spermatozoa obtained from the excurrent ducts were comparable with the concentrations of spermatozoa in samples obtained via dorso-abdominal massage method.

Sperm Motility

Table 1 presents the motility of spermatozoa retrieved using SUM and MM. As shown on the table, the motility of samples retrieved using SUM was 65.5 ± 4.97% and 65 ± 4.71% for MM-retrieved samples. T-test revealed no significant difference between the motility of the SUM-retrieved and MM-retrieved spermatozoa indicating that the retrieval method did not significantly affect or improve the resulting percentage of motile and progressive spermatozoa in the sample collected. Higher sperm motility has been observed in fresh ejaculated semen of cocks (66.67 ± 6.67% to 86.5± 0.78%) as reported in earlier studies [16, 17, 18, 19, 20, 21].

Table 1. Comparison of various parameters of spermatozoa retrieved via swim-up and mincing techniques

Retrieval Method	Semen Parameters			
	Concentration (x 10^9)	Motility (%)	Viability (%)	Abnormality (%)
SUM	2.08 ± 1.1	65.5 ± 4.97	85.65 ± 7.88	2.49 ± 1.83
MM	1.93 ± 1.17	65 ± 4.71	84.20 ± 8.79	4.11 ± 3.50

Values were expressed as mean ± SD. Results were analyzed using t-test for two independent samples.

In a study conducted [22], a gradual increase in sperm motility was observed from the spermatozoa retrieved from different regions of the excurrent ducts beginning from the epididymal region to the distal part of the ductus deferens that corresponds to the degree of sperm maturation. The increasing capacity for motility was attributed to the acquisition of power of movement by the sperm as it passed through the excurrent ducts. Also, it was indicated that the motility of spermatozoa obtained from the distal ductus deferens was comparable to that of ejaculated spermatozoa.
The relatively lower percent motility observed from the samples obtained from the excurrent ducts could therefore be explained by the presence of immature spermatozoa from proximal region of the duct that were mixed with the mature and highly motile spermatozoa of the distal part thereby affecting the mass motility.

Sperm Viability

Percentage of live spermatozoa in the sample retrieved by SUM was found to be 85.65 ± 7.88% whereas for MM-retrieved spermatozoa, viability was 84.20 ± 8.79. Statistical analysis showed no significant difference between the viability of the SUM- and MM-retrieved spermatozoa. Results indicated that the retrieval method had no effect on the resulting percentage of viable sperm in the sample.
The percentage of viable spermatozoa from fresh ejaculated samples of various breeds or strains observed in earlier studies ranged from 80.3 ± 0.9% to 89.63 ± 1.32% [18, 19, 20]. The viability of samples obtained in this study was comparable to the results of these earlier studies. In domestic fowl, the spermatozoa undergo maturation and become fully fertile in the ductus deferens. It is also in the ductus deferens where spermatozoa produced by the testes are stored until the rooster mates. It was therefore expected that the percentage of live spermatozoa in the excurrent ducts would resemble that of ejaculated samples.

Sperm Abnormality

Almost always, some spermatozoa from an ejaculate exhibit various forms of deviation from the normal morphology that, when present in great proportion, may adversely affect fertility [23]. Figure 1 showed some of the abnormalities observed in the samples isolated from the excurrent ducts, which included coiled head, bent head, no tail, coiled tail, and bent midpiece.

As presented in Table 1, the percentage of morphologically abnormal spermatozoa in samples retrieved by SUM was found to be 2.49 ± 1.83% while 4.11 ± 3.50% abnormality was observed in MM-retrieved samples. No significant difference was found between the percentages of abnormality of spermatozoa retrieved using SUM and MM. Table 2 shows the percentages of abnormality in the head, midpiece and tail region of obtained samples. No region in particular showed a greater percentage of defects.

The percentages of abnormal spermatozoa observed from both of the samples retrieved from the excurrent ducts were lower than those observed from ejaculated spermatozoa of various strains in studies conducted [16, 18,19, 20, 21] which ranged from 4.52 ± 10% to 23.33 ± 6.67%.
Percent normal spermatozoa had been identified [2] as one of the most reliable indicators of suitability of samples for cryopreservation. Samples retrieved from the excurrent ducts

had a relatively lower percentage of abnormal spermatozoa as compared to fresh ejaculated samples of other breeds or strains. Although this may be attributed to the genetic or intrinsic factors of Philippine local chicken, unfortunately, literatures regarding characterization of semen ejaculates of local breeds or strains are lacking.

The results of the study proved that good quality semen samples could be collected from the excurrent ducts of chicken with parameters that were comparable to ejaculated sperm of some strains. Samples retrieved from the excurrent ducts could be considered as better source for future rooster semen studies regarding the development of methods of chicken sperm cryopreservation and, eventually, in cryopreservation per se of valuable genetic resources.

Table 2. Comparison of total morphological defect rates of head, midpiece and tail of spermatozoa retrieved by swim-up and mincing methods

Retrieval Method	Sperm abnormalities		
	Head (%)	Midpiece (%)	Tail (%)
SUM	1.14 ± 1.24	0.33 ± 0.46	1.02 ± 0.89
MM	1.63 ± 2.13	0.62 ± 0.56	1.86 ± 2.43

Values were expressed as mean± SD. Results were analysed using one-way ANOVA.

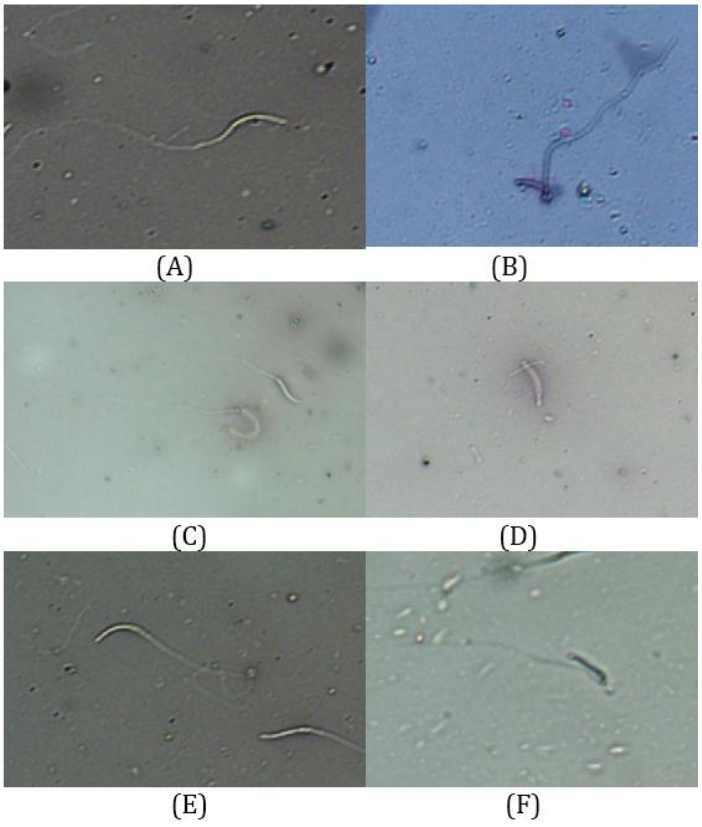

Figure 1. Photomicrography of normal and abnormal morphology of cock spermatozoa under HPO. (A) morphologically normal, (B) coiled head, (C) bent head, (D) no tail, (E) coiled tail, (F) bent midpiece.

CONCLUSIONS AND RECOMMENDATIONS

In this study, the isolation of spermatozoa from the excurrent ducts of Philippine local chicken was made possible and also the difference between the quality of spermatozoa retrieved by mincing and swim-up methods was studied. The study clearly proved that good quality spermatozoa could be collected from the excurrent ducts of chicken. Many studies, however, have shown that various factors, which include species, age, weight and others, play significant roles in the differences in semen parameters. As such, a similar study that is species-specific or strain-specific for Philippine local chickens is recommended. Moreover, other in vitro tests and subsequent correlation with cryosurvivability are suggested.

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COMPETING INTEREST

The authors have declared that no competing interests exist.

REFERENCES

1. Woelders H, Zuidberg CA and Hiemstra SJ. Animal Genetic Resources Conservation in the Netherlands and Europe: Poultry Perspective. Poultry Science 2006, 85: 216-222.
2. Blesbois E. Current status in avian semen cryopreservation. World's Poultry Science Journal, 2007, 63, 213-222. doi: 0.1017/S0043933907001419.
3. Sarabia AS and Cruz LC. Cryobanking of animal genetic resources: The Philippine experience. Retrieved from www.angrin.tlri.gov.tw. 2008.
4. Roushdy K, El-Sherbieny MA, Abd El-Gany FA and El-Sayed MA. Semen cryopreservation for two local chicken Strains as a tool for conservation of Egyptian Local genetic resources. Egyptian Poultry Science Journal 2014, 34:607-618.
5. Tisdell C. Socioeconomic causes of loss of animal genetic biodiversity: analysis and assessment. Ecol Econ 2003, 45: 365–376.
6. Purdy, P. H. "A review on goat sperm cryopreservation." *Small Ruminant Research*, 2006, 63(3), 215-225.
7. Łukaszewicz E, Jerysz A, Partyka A and Siudzinska A. Efficacy of evaluation of rooster sperm morphology using different staining methods. Research in Veterinary Science 2008. doi:10.1016/j.rvsc.2008.03.010.
8. Gerzilov V. Influence of various cryoprotectants on the sperm mobility of Muscovy semen before and after cryopreservation. Agricultural Science and Technology 2010, 2(2):57-60.
9. Chelmonska B, Jerysz A, Lukaszewicz E, Kowalczyk A and Malecki I. Semen collection from Japanese quail (*Coturnix japonica*) using a teaser female.

Turkish Journal of Veterinary and Animal Sciences 2008, 32(1):19-24.
10. Blanco JM, Gee GF, Wildt DE and Donoghue AM. Producing progeny from endangered birds of prey:Treatment of urine-contaminated semen and a novel intra-magnal insemination approach. Journal of Zoo and Wildlife Medicine 2002, 33(1): 1-7.doi:10.1638/1042-7260 (2002) 033[0001: PPFEBO] 2.0.CO.2.
11. David G. Antalan III, Flocerfida P. Aquino, Ma. Elizabeth DC. Leoveras, Lerma C. Ocampo, Eufrocina P. Atabay and Angeles M. De Leon, The effect of semen extender and storage time on the quality of spermatozoa collected from the excurrent duct of Philippine local chicken, Journal of Biological Engineering Research and Review*, 2015, 2(2), 18-20.*
12. Beltran MAG. Cryopreservation of goat semen for A.I. Knowledge Resource Management Center, Philippine Carabao Center 2011.
13. 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, Philippines 2004.
14. Glover TD. Mating males: An evolutionary perspective on mammalian reproduction. Cambridge: Cambridge University Press 2012.
15. Hicks KD. Ratite reproduction. Proceedings of the Annual Conference of the Association of Avian Veterinarians 1992, 318-325.
16. Ajayi FO, Agaviezor BO and Ajuogo PK. Semen characteristics of three strains of local cocks in the humid tropical environment of Nigeria. International Journal of Animal and Veterinary Advances 2011, *3*(3): 125-127.
17. Almahdi AB and Ondho YS. Comparative studies of semen quality on different breeds of chicken in Poultry Breeding Center, Temanggung-Central Java. International Refereed Journal of Engineering and Science 2014, *3*(2): 94-103.
18. Churchil RR, Praveena PE, and Sharma D. Semen quality parameters, their inter-relationship and post-washing sperm attributes of Rhode Island Red rooster. Veterinary World 2014, 7(12): 1117-1122.
19. Tabatabaei S, Batavani RA, and Talebi AR. Comparison of semen quality in indigenous and Ross broiler breeder roosters. Journal of Animal and Veterinary Advances 2009, 8(1): 90-93.
20. Tarif A, Bhuiyan M, Ferdousy R, Juyens N and Mollah M. Evaluation of semen quality among four chicken lines. IOSR Journal of Agriculture and Veterinary Science 2013, 6(5): 7-13.
21. Tuncer PB, Kinet H and Ozdogan N. Evaluation of some spermatological characteristics in Gerze cocks. Veterinary Journal of Ankara University 2008, 55: 99-102.
22. Ahammad MU, Nishino C, Tatemoto H, Okura N, Kawamoto Y, Okamoto S and Nakada T. Maturational changes in motility, acrosomal proteolytic activity, and penetrability of the inner perivitelline layer of fowl sperm, during their passage through the male genital tract. Theriogenology, 2011, 76:1100-1109.
23. Basu SC. Male reproductive dysfunction. New Delhi: Jaypee Brothers 2005.

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Vicky B. Salting is a consistent honor student during her primary and secondary education. She likewise finished her Baccalaureate degree in Biology, Cum Laude, at the Central Luzon State University. After her graduation in April 2015, she was hired as Research Assistant at the Animal Health Unit of the Philippine Carabao Center where she currently works on the "Evaluation of ovicidal action of nematode predacious fungus *Pochonia chlamydosporia* against Fasciola sp. in water buffaloes (Bubalus bubalis).