 KHON KAEN AGR. J. 43 SUPPL. 2 : (2015).

Effects of Sericin Supplementation on the Sperm Survival of Cooled Stored Chicken Semen

Pronjit Sonseeda[3], Sannampoung Somtao[1], Banyat Laophaiboon[2], Monchai Duangjinda[1,2] and Thevin Vongpralub[1,2*]

ABSTRACT: Silkworm (*Bombyxmori*) protein, sericin, is water soluble protein, known as an effective antioxidant and antimicrobial. The present study was conducted to determine the effect of sericin on quality of cooled storage chicken semen. Semen samples from Thai native chicken were diluted with IGGKPh diluent (control) or supplemented with sericin 0.025, 0.05, 0.1 and 0.2% and stored at 5ºC for 5days. The vigor score of motility, progressive motile and live sperm were evaluated as parameter for semen quality. Result showed that sericin supplementation into the extender gave beneficial to sperm vigor and progressive motile and live sperm compared to control ($p<0.05$). The finding of the present study suggested that the addition of sericin at 0.025% can be recommended as a component of chicken semen extender for liquid storage.
Keywords: Liquid storage, Silkworm protein, Chicken semen

Introduction

In broiler industry, selection of broad-breasted male strain has led to face with decline in fertility of flock mating. Similar to the turkey industry, if male chicken size become large that cause mating problem, then artificial insemination may play a crucial role applying in chicken production. Due to the high susceptibility of chicken spermatozoa to damage during stored, most artificial insemination are conducted using stored semen preserved at the refrigerated temperature up to 24 h in chicken in which fertility obtains similar to fresh semen. When storage time is more than 24 h in vitro, semen fertility significant decreased (Donoghue and Whishart, 2000). Chicken sperm membrane is abundance in polyunsaturated fatty acid, which cause cell very susceptible to the harmful effect of reactive oxygen species (Fujihara and Koga, 1984). The production of toxic reactive oxygen species during *in vitro* storage of semen can cause loss in sperm motility and viability (Aitken and Clarkson, 1988). Sericin, a water soluble protein derived from silkworm (*Bombyx-mori*) has been reported on it antioxidant properties (Isobe et al., 2012; Takechi et al., 2014), antibacterial activity and act as cryoprotective agent for bovine embryo (Isobe et al, 2013) and for buffalo semen. Kumar et al., (2015) report that sericin improves frozen-thawed semen quality through protecting sperm from oxidative stress. Due to its antioxidant and antibacterial properties, sericin could be added into long term semen extender in order to improve the quality of cooled semen.

[1] Department of Animal Science, Faculty of Agriculture, Khonkaen University, Khon Kaen, Thailand 40002
[2] Research and Development Network Center for Animal Breeding (Native Chicken) Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand 40002
[3] Faculty of Agricultural Technology, Nakhon Phanom University, Nakhon Phanom Thailand 48000
* Corresponding author: vthevi@kku.ac.th

However, effect of sericin on storage of cooled chicken semen has never been studied. Therefore, the objective of this study was to investigate whether the addition of diluent with sericin could improve the quality of diluted chicken semen stored at 5^0C for 5 days.

Material and Method

Animals and treatments

Twenty four native cocks (Pradu Hang Dam, 2 years of age) were housed in individual cage in an open-house system and assigned to four groups. The animals were fed with 130 g/d/b and water *ad libitum* and reared under natural environment condition where they received a natural light: dark photoperiod (14.00 L: 10.00D) throughout the experiment.

Semen collection , assessment and preparation

Semen was collected by massage method squeezing of the region surrounding the side of the cloaca to obtain semen as described by Lake and Stewart (1978). Semen from an individual cock was collected in a 1.5 ml micro tube containing 0.1 ml. with IGGKPh diluent (compose of potassium citrate 0.14 g, sodium glutamate 1.4g, dipotassium hydrogen phosphate 0.98 g, sodium dihydrogen phosphate0.21 g, glucose 0.9 g inosital 0.9 g, dissolved in double-distilled water 100 ml). Semen collection was always performed by the same person and special care was taken to avoid contamination of semen with feces, uret and transparent fluid, which lower semen quality. Immediately after collection, sperm motility was evaluated under microscope, semen samples with sperm motility at least 80% were used in this study. Semen samples from each male group were pooled in 15 ml centrifuge tube, then each sample was divided to five aliquots and diluted 1:4 (semen: diluent) with IGGKPh diluent supplemented with sericin according to treatments(0, 0.025,0.05, 0.01 and 0.02% sericin). Subsequently, each sample was divided to three aliquots in 1.5ml microtube and times of evaluation (1, 3and 5 day of storage). The concentration of sperm was assessed by Neubauer hemocytometer.

Evaluation of stored semen quality

Vigor motility score (0-5) assessment using an arbitrary scale of 0-5(0 being no movement, 5 being a very strong movement). A drop of 15 microliters of semen was dropped on warmed slide under microscope at 40x magnification.

For progressive motility, 15 microliters of diluted semen was dropped on cleaned wormed slide and covered with cover slip and examined under microscope at 400x magnification. Motility was expressed as percentage of progressive motile spermatozoa.

The viability of sperm was evaluated by fluorescence staining (Live/dead sperm viability kit, 7011 Invitrogen, USA). Sperm motility and viability assessment were carried on at day 1, 3 and 5 of storage.

Statistical analysis

The experiments were conducted as randomized complete block design (RCBD) and statistical analysis of data was performed by General Linear Model (GLM) procedure of the SAS (1996). The mean of the effect of treatment were compared using Duncan's Multiple Rang test (DMRT) difference were considered to be significant when $P<0.05$.

Result and Discussion

Motility score, percentage of progressive motile, percentage of live sperm and sperm concentration of raw semen samples using in this study were approximately 4.53, 93.3%, 97.8% and 4,845.8 cell/ml, respectively.

The sperm motility vigor score (0-5), percentage of motile sperm and percentage of live sperm of chicken semen were assessed at 1, 3, and 5 day after storage at 5°C. The results are presented in **Table 1**. The results show that supplementation of sericin (0.025-0.2 %) in diluent improved vigor of sperm movement and motility at day 5 of storage. For the percentage of live sperm, at 1 day after storage, sericin at 0.025% was beneficial to sperm viability in cooled chicken semen, compared to control ($p<0.05$).

Table 1 Effect of sericin supplementation into extender on semen quality of native chicken spermatozoa during 1, 3 and 5 days of storage (mean ± SD)

Treatment	1 day	3 day	5 day
Mass movement			
Control	4.37^{a}±0.25	3.15^{a}±0.29	3.02^{b}±0.15
0.025	4.50^{a}±0.01	3.25^{a}±0.41	3.12^{a}±0.22
0.05	4.62^{a}±0.25	3.32^{a}±0.50	3.15^{a}±0.25
0.1	4.50^{a}±0.41	3.25^{a}±0.42	3.17^{a}±0.26
0.2	4.37^{a}±0.25	3.17^{a}±0.30	3.15^{a}±0.24
Motility			
Control	90.0^{a}±1.83	76.2^{a}±11.09	67.5^{b}±8.66
0.025	89.2^{a}±1.71	77.0^{a}±11.52	72.5^{a}±8.66
0.05	90.7^{a}±1.89	78.5^{a}±12.50	75.0^{a}±10.80
0.1	89.5^{a}±1.73	77.0^{a}±11.52	74.0^{a}±10.23
0.2	90.0^{a}±1.41	76.2^{a}±11.09	72.5^{a}±8.66
Viability			
Control	86.9^{b}±2.71	86.9^{b}±2.71	86.9^{b}±2.71
0.025	91.8^{a}±2.15	91.8^{a}±2.15	91.8^{a}±2.15
0.05	90.2^{ab}±2.11	90.2^{ab}±2.11	90.2^{ab}±2.11
0.1	90.5^{ab}±0.83	90.5^{ab}±0.83	90.5^{ab}±0.83
0.2	87.0^{b}±3.11	87.0^{b}±3.11	87.0^{b}±3.11

Different letters (^{a,b}) within columns indicate significant differences ($P<0.05$)

These results are supported by the finding of Kumar el al.,(2015) where sericin in the extender improves the quality of cryopreserved buffalo semen by protecting sperm against oxidative stress. The addition of sericin to bovine in vitro culture medium was also improved embryo development by preventing H_2O oxidative stress (Isobe et al., 2012). Morever, this silkworm protein was also had antimicrobial property (Sarovart et al.,2003; Pushpa et al., 2013). Liquid storage has been reported to induce chicken spermatozoa decreased motility and viability by oxidative stress(Donoghue and Wishart, 2000). In the present study, the addition of sericin enhanced the quality of liquid storage of chicken semen. The improvement in the chicken stored semen of this protein from silkworm may be by it antioxidant and antimicrobial property. However, the further investigation on the properties of this protein on improving of chicken storage semen and application in AI in this species are needed.

References

Aitken, R.J. and J. S. Clankson. 1988. Significant of reactive oxygen species and antioxidationsin defining the efficacy of sperm preparation techniques. J. Androl. 9:367-376.

Donoghue, A.M., G.J. Wishart. 2000. Storage of poultry semen. Anim. Reprod.Sci.62: 213-232.

Fujihara, N., O. Koga. 1984. Prevention of the production of lipid peroxide in rooster spermatozoa. Anim. Reprod. Sci.7: 385-390.

Isobe,T., Y. Iketaba, T. Onitsuka, M. Wittayarat, Y. Sato, M. Taniguchi and T. Otoi. 2012. Effect of sericin on preimpantation development of bovine embryos cultured individually. Theriogenology 23:318-325.

Isobe, T. Y. Ikebata, T. Onitsuka, L.T. Do, Y. Sato, M. Taniguchi and T. Otoi. 2013. Cryopreservation for bovine embryos in serum –free freezing medium containing silk protein sericin. Cryobiology. 67:184-187.

Kumar, P., D. Kumar, P. Sikka and P. Singh,. 2015. Sericin supplementation improves semen freeezability of buffalo bulls by minimizing oxidative stress during cryopreservation. Anim. Reprod. Sci. 152:23-31.

Lake, P.E. and J.M. Stewart. 1978. Preservation of fowl semen in liquid nitrogen-an improved method. Br.Poult. Sci. 19:187-194.

Pushpa, A., A.V.G. Vishnu and K.S. Tippa Reddy 2013. Preparation of nano silk sericin based hygrogels from silk industry waste. J. Environ.Res.Dev. 8: 243-253.

Sarovant, S., B. Sudatis, P. Meesilpa, B.P. Gvandy and R. Magaraphan.2003. The use of sericin as an antioxidantand antimicrobial for polluted air treatment. Rev.Adv.Mater. Sci. 5:193-198.

Takechi,T., R. Wada, T. Fukuda, K. Harada and H. Takamura. 2014. Antioxidant activities of two sericin proteins extend from cacoon of silkworm (*Bombyx-mori*) measured by DPPH, Chemiluminescence, ORAC and ESR methods. Biomed.Pep. 2:364-369.