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### Research article

## The sperm longevity and freezability in the modified BHSV extender of Thai Pradu-hangdum chicken

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### Abstract

Pradu-hangdum is a distinct Thai native chicken. To conserve its purebred genetics, artificial insemination has been explored but this technique requires the selection of good quality semen. Hence, the objective of this study was to characterize the sperm of Pradu-hangdum and their longevity and freezability in the BHSV extender. Semen samples from 30 randomly selected roosters were collected and examined macroscopically and microscopically to identify semen characteristics. Results revealed that the Pradu-hangdum sperm had a spiral-shaped head, mid-piece and tail and the semen was white cream in color. The average sperm concentration was  $5.24 \times 10^9 \pm 0.58$  sperm/mL and the mean volume was  $0.22 \pm 0.03$  mL. Mean total sperm motility was highest when stored in BHSV extender at 1 h at 85.20%, 56.00% at 24 h and 36.33% at 48 h, respectively. The longevity of stored sperm decreased significantly at 24 h and 48 h ( $P < 0.05$ ). The freezability of Pradu-hangdum semen also showed a significant decline from 81.45% to 57.02% sperm motility ( $P < 0.05$ ) but still within the acceptable range of sperm motility and kinetic parameters for insemination. In conclusion, these results indicate that semen stored for 24 h or frozen in BHSV extender can be available for use in actual practice.

**Keywords:** Freezability, Longevity, Pradu-hangdum chicken, Semen quality, Sperm morphology

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## INTRODUCTION

Pradu-hangdum is a pure breed of Thai native chicken that has a special characteristic taste of meat, can adapt to the weather and is resistant to infectious harmful diseases (Kammongkun and Leotaragul, 2015; Sawasdee, 2015). For two decades, the Thai government has been trying to preserve their pure genetics by encouraging farmers to keep them for meat production. However, the low fertility rate due to the ratio of male to female and unmanaged crossbreeding were the major problems in natural insemination resulting in their low population (Kammongkun and Leotaragul, 2015).

Artificial insemination (AI) is an important technique in the conservation of indigenous avian species (Blanco et al., 2009) wherein the semen from one male chicken breeder could be shared with many other female breeders. While the method is cheap and advantageous, the quality of the semen is the key to success in using AI (Mohan et al., 2018). Basically, fresh semen directly obtained from a male is more effective than frozen semen (Mitchell et al., 1977). The time duration of semen collection and the AI process vary from 1 h to more than 24 h due to several factors leading to the low success rate of AI.

Aside from cryopreservation, biotechnologists have not yet been able to develop other methods of long-term sperm storage in in-vitro conditions. In contrast to mammalian species, cryopreservation is not a reliable approach for storing avian germ cells due to the low fertilizing ability of frozen-thawed sperm (Long, 2006; Long et al., 2014) owing to the differences in sperm shape, membrane fluidity and high amounts of polyunsaturated fatty acids present in the avian sperm (Çiftci and Aygun, 2018). Avian sperm plasma membrane becomes damaged and spermatozoa abnormal after the process (Bakst and Sexton, 1979). Cryopreservation injures avian sperm resulting in decreased sperm viability and membrane integrity which are the major limitations of avian sperm storage (Blesbois et al., 2005).

The Blumberger Hahnen Sperma Verdünner (BHSV) extender was adapted to prolong storage and cryopreserve fresh semen (Seigneurin et al., 2013). by protecting sperm against possible damage brought about by toxic seminal plasma, providing nutrients, cooling buffers, and preventing bacterial growth. Despite the BHSV extender is a simple preparation, the most popular extender used for turkey semen storage is the Beltsville Poultry Semen Extender (BPSE). Such extenders are buffers that promote the immediate survival of spermatozoa because they provide osmolarity (330–400 mOsm) and pH (7.0–7.5) similar to that of the seminal plasma. However, data on Pradu-hangdum sperm longevity and freezability in BHSV extender are still unknown. Thus, this study characterized the sperm of Pradu-hangdum chicken by morphology examination and determined their longevity and freezability in BHSV extender.

## MATERIALS and METHODS

### Experimental animals

Thirty (30) 1-year-old male purebred Pradu-hangdum chickens with phenotypic characteristics of the breed and weighing an average of 3.5 kg were used in the experiment (Kammongkun and Leotaragul, 2015). The chickens

were housed individually in a high-raised cage (120 cm ×120 cm ×120 cm) exposed to natural light at 12 h per day. Feed containing 17% protein (Betagro, Lamphun, Thailand) were given to the chickens at approximately 200 g/chicken/day with an ad libitum supply of water. All experiments were conducted in accordance with the Institutional Animal Care and Use Committee guidelines of the Faculty of Veterinary Medicine, Chiang Mai University under approved protocol no. S33/2561.

### **Semen collection**

Semen was collected in a 1.5 mL sterile centrifuge tube (Eppendorf, Hamburg, Germany) in the morning once a week for 3 times using dorso-abdominal massage (Burrows and Quinn, 1937). The semen was subsequently stored in BHSV extender (glucose 0.5 g, sodium glutamate 2.85 g, potassium acetate 0.5 g, magnesium acetate 0.07 g, myo-inositol 0.25 g and ultrapure water 100 mL, pH 7.15, osmolarity 380 mOsm) and kept at 5 °C until use (Seigneurin et al., 2013).

### **Fresh semen analysis**

Macroscopic examination of semen color and volume was performed and their microscopic characteristics were then evaluated. Sperm concentration (sperm/mL), and percentage of total motility were recorded. The diluted semen was smeared on a glass slide and stained with eosin-nigrosin. Sperm morphology and viability were observed under a light microscope (Leica, Wetzlar, Germany).

### **Sperm longevity**

Chicken semen was diluted with BHSV extender in the ratio of 1:2 to 1:4 up to the desired concentration. The diluted semen was stored at 5 °C, and sperm longevity of individual semen (n=90) was analyzed using total sperm motility which was observed under the microscope after 1 h, 24 h, and 48 h of being stored in BHSV extender.

### **Sperm freezability**

Individual semen (N=90) was diluted with BHSV extender and equilibrated at room temperature (25 °C) for 30 min and at 5 °C for 5 min (Seigneurin et al., 2013). 10 % (v/v) Ethylene glycol (EG) was added into the semen extender and mixed with the semen. The mixture was equilibrated at 5 °C for 15 min. For the freezing process, the semen was prepared in 0.25 mL straw tubes (Minitube GmbH, Tiefenbach, Germany) and the straws were placed at approximately -120 °C (over the liquid nitrogen vapor) for 10 min, plunged into the liquid nitrogen and kept in liquid nitrogen for 1 week. The frozen semen was separately thawed for 15 sec at 40 °C before analysis. Fresh and frozen-thawed sperm motion and kinetic parameters were evaluated using computer-assisted semen analysis (CASA, Hamilton Thorn Motility Analyzer, IVOS12.3, USA).

## Statistical analysis

The mean and standard error of mean (SEM) of semen volume, concentration, percentage of normal morphology and percentage of viability were reported using descriptive analysis. For longevity, the mean total motility was compared between storage times with ANOVA while motion parameters and sperm kinetics were analyzed between fresh and frozen semen using the standard T-test by Graph Pad prism software (GraphPad Software Inc., San Diego, USA). The significant difference was accepted at  $P$ -value  $< 0.05$ .

## RESULTS

### Fresh semen quality

After the macroscopic evaluation of the semen, all samples presented white cream color and normal pH. The semen volume ranges between 0.08 – 0.50 mL (average  $0.215 \pm 0.08$  mL) while the mean sperm concentration was  $5.24 \times 10^9 \pm 0.58$  sperm/mL (Table 1). Morphological examination under the microscope revealed the spiral-shaped heads of the spermatozoa, which were difficult to distinguish from the midpiece and long tails. The percentage of normal sperm was  $88.68 \pm 0.54$  and the mean percentage of viable sperm was  $83.58 \pm 1.16$ .

**Table 1** Volume and concentration of Pradu-hangdum chicken's semen (n =90).

Time of collection	Volume (mL)		Concentration ( $\times 10^9$ cells/mL)	
	Mean	SEM	Mean	SEM
1	0.235	0.032	7.71	0.77
2	0.209	0.033	4.39	0.52
3	0.201	0.026	3.63	0.45
Total	0.215	0.030	5.24	0.58

### Sperm motility and longevity analysis

For fresh semen quality, the mean total motility was  $91.13 \pm 1.08\%$ , the progressive motility was  $77.13 \pm 0.81\%$  and the mean mass motility score was  $3.93 \pm 0.28$ . After 1 h, 24 h, and 48 h of storage in the BHSV extender, the longevity of semen as evaluated by mean total sperm motility percentage was at  $77.13 \pm 7.73\%$ ,  $56.00 \pm 7.25\%$ , and  $36.33 \pm 7.44\%$ , respectively (Table 2). This total sperm motility data was at a normal distribution. However, student T-test was used to analyze and compare the two groups due to the homogeneity of variance of these data. The total sperm motility of semen stored for 24 h or 48 h in the BHSV extender decreased significantly compared to 1 h of storage ( $P < 0.05$ ). In addition, a significant decrease in sperm motility was also noted from 48 h to 24 h storage with extender ( $P < 0.05$ ).

**Table 2** Longevity of Pradu-hangdum chicken's semen storage at 4°C in different time (n = 30).

Time after collection	Total motility (%)	
	Mean	SEM
1 h	77.13 <sup>a</sup>	7.73
24 h	56.00 <sup>b</sup>	7.25
48 h	36.33 <sup>c</sup>	7.44

a, b and c mean significant different was accepted at  $P < 0.05$

### Comparison of sperm motion and kinetic parameters between fresh and frozen-thawed semen

The motion and kinetic parameters were shown in [Table 3](#), [Table 4](#) and [Table 5](#). Results revealed that fresh semen, with regards to the sperm motion parameters, was significantly better than the frozen-thawed semen ( $P < 0.05$ ). In addition, the sperm kinetic parameters from fresh semen particularly VCL (velocity curve line), VSL (velocity straight line), VAP (average path velocity), DCL (distance curve line), DSL (distance straight line), DAP (distance average path), and ALH (amplitude of lateral head movement), BCF (beat-cross frequency), and HAC (head activity) were significantly higher than those from frozen-thawed semen. In contrast, some kinetic parameters such as WOB (wobble), LIN (linearity), and STR (straightness) showed no significant difference between the fresh and frozen-thawed semen. Individual chicken sperm motion parameter was highlighted in [Figure 1](#) but none of them exerted individual effects on each sperm in this study. Moreover, none among the sperm kinetic parameters that showed significant differences between fresh and frozen-thawed semen had individual effects on each sperm except DCL ([Figure 2](#)).

**Table 3** Comparison of mean of sperm motion parameters between fresh semen and frozen-thawed semen of Pradu-Hangdam chicken.

Type of semen	TM		PM		PFM		PSM	
	Mean (%)	SEM	Mean (%)	SEM	Mean (%)	SEM	Mean (%)	SEM
<b>Fresh</b>	81.45 <sup>a</sup>	1.90	67.08 <sup>a</sup>	1.82	31.91 <sup>a</sup>	1.54	35.13 <sup>a</sup>	0.80
<b>Frozen</b>	57.02 <sup>b</sup>	1.03	34.17 <sup>b</sup>	0.94	12.86 <sup>b</sup>	0.44	21.30 <sup>b</sup>	0.60

TM= Total motility, PM= Progressive fast motility, and PSM= Progressive slow motility Mean with different superscript letters indicate significant differences ( $P < 0.05$ ).

**Table 4** Comparison of sperm kinetic parameter between fresh semen and frozen-thawed semen of Pradu-Hangdam chicken.

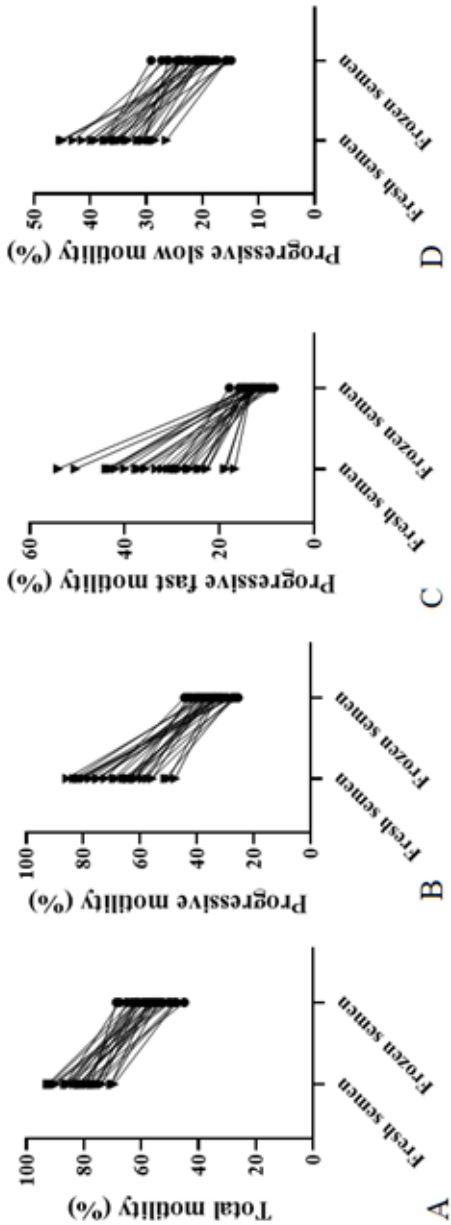
Type of semen	VCL		VSL		VAP		DCL		DSL		DAP		ALH	
	Mean ( $\mu\text{m/s}$ )	SEM	Mean ( $\mu\text{m/s}$ )	SEM	Mean ( $\mu\text{m/s}$ )	SEM	Mean ( $\mu\text{m/s}$ )	SEM	Mean ( $\mu\text{m/s}$ )	SEM	Mean ( $\mu\text{m/s}$ )	SEM	Mean ( $\mu\text{m/s}$ )	SEM
<b>Fresh</b>	103.7 <sup>a</sup>	3.04	37.94 <sup>a</sup>	1.43	49.06 <sup>a</sup>	1.54	21.77 <sup>a</sup>	0.62	4.93 <sup>a</sup>	0.15	8.28 <sup>a</sup>	0.22	1.03 <sup>a</sup>	0.03
<b>Frozen</b>	57.97 <sup>b</sup>	1.40	21.33 <sup>b</sup>	0.52	27.59 <sup>b</sup>	0.62	15.42 <sup>b</sup>	0.39	2.74 <sup>b</sup>	0.08	5.00 <sup>b</sup>	0.13	0.61 <sup>b</sup>	0.01

VCL=Velocity curve line, VSL=Velocity straight line, VAP=Average path velocity, DCL= Distance curved line, DSL= Distant straight line, DAP=Distance average path, and ALH=amplitude of lateral head movement Mean with different superscript letters indicate significant differences ( $P < 0.05$ ).

**Table 5** Comparison of sperm kinetic parameter between fresh semen and freezing semen of Pradu-Hangdam chicken

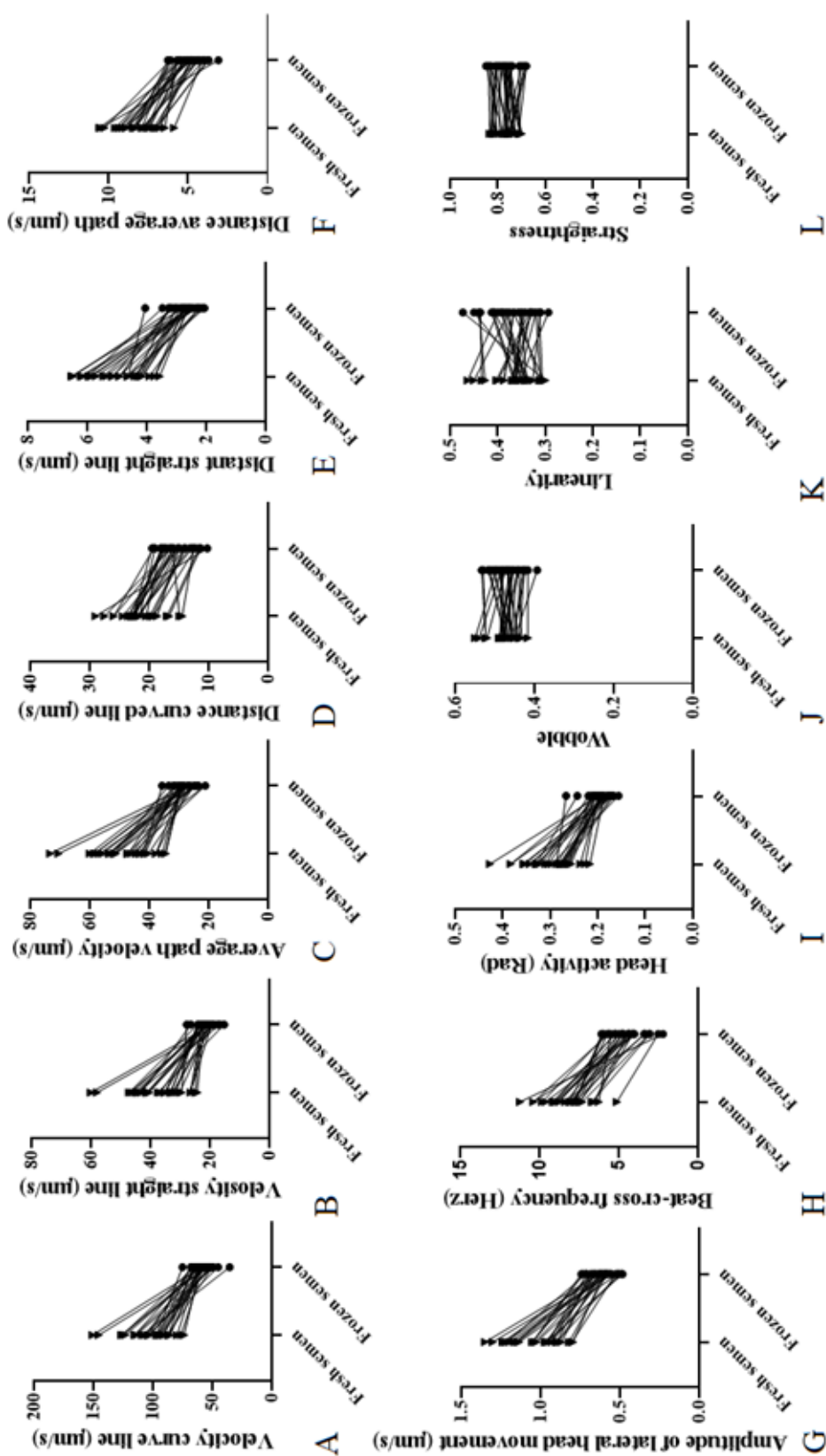
Type of semen	BCF		HAC		WOB		LIN		STR	
	Mean (Herz)	SEM	Mean (Rad)	SEM	Mean (VAP/VCL)	SEM	Mean (VSL/VAP)	SEM	Mean (VSL/VAP)	SEM
Fresh	8.23 <sup>a</sup>	0.23	0.30 <sup>a</sup>	0.01	0.47 <sup>a</sup>	0.06	0.36 <sup>a</sup>	0.01	0.76 <sup>a</sup>	0.01
Frozen	4.50 <sup>b</sup>	0.16	0.19 <sup>b</sup>	0.01	0.47 <sup>a</sup>	0.01	0.37 <sup>a</sup>	0.01	0.77 <sup>a</sup>	0.01

BCF=Beat-cross frequency, HAC=Head activity, WOB=wobble, LIN=Linearity, and STR=Straightness Mean with different superscript letters indicate significant differences (P < 0.05).



**Figure 1** The individual of sperm motion parameter between fresh semen and frozen-thawed semen of Pradu-Hangdam chicken.





**Figure 2** The individual of sperm kinetic parameter between fresh semen and freezing semen of Pradu-Hangdam chicken.



## DISCUSSION

Determination of the motility or viability of spermatozoa after semen collection is important for artificial insemination. Poor quality semen could be a major cause of low fertility rate and high embryo mortality rate (Donoghue and Wishart, 2000). Routine semen evaluation procedures include determination of semen volume, color, concentration, motility, viability and morphology of spermatozoa. Besides, these assessments on fresh semen showed correlation with the success of AI. The present study provides basic knowledge of semen evaluation in Pradu-hangdum chicken. Semen was collected by the dorso-abdominal massage technique, and the longevity of sperm in extender at different time points was evaluated. The morphology of Pradu-hangdum chicken sperm was similar as with other breeds of chicken as illustrated in previous studies (Abu Md Mamun et al., 2013; Feyisa et al., 2018). The mean concentration of fresh semen in this study was higher than that of Arabic male chickens at  $2.20 \pm 0.37$  billion sperm / mL (Almahdi et al., 2014). Meanwhile, the sperm volume in the present study was comparable to other avians (Gee et al., 2004).

Although sperm survive at the body temperature of 41 °C in both male and female reproductive tracts, this ability is lost within a few hours when sperm are incubated at 41 °C in vitro. Generally, sperm motility is low at both high and low temperatures and high in temperatures between 20-37 °C. However, in sperm viability studies, a high percentage of viable sperm is usually found at low temperatures (approximately 4-5 °C). In this study, it was found that sperm storage in BHSV extender at 5 °C for 24 h yielded an acceptable motility data. This is possibly due to the decreased metabolic activity of spermatozoa at low temperatures which might also be associated with the long-term sperm viability in vitro condition. Storage of turkey fresh semen at 5 °C for 18 h provides similar fertility but this decreased with storage at 15 °C, 25 °C and 35 °C (Giesen and Sexton, 1983). Important to the development of diluents and storage systems for poultry semen are the physiological differences and metabolic requirements of spermatozoa from different species. Chicken spermatozoa are metabolically competent in both aerobic and anaerobic environments in vitro. Semen diluents are based on the biochemical composition of chicken and turkey semen (Lake, 1995). Hence, glutamic acid, the most prominent anionic constituent of avian seminal plasma, became a standard component of these diluents. However, Seigneurin et al. (Seigneurin et al., 2013) found that BHSV extender could increase the fertility rate of frozen-thawed sperm and is, therefore, better than other extenders because of the myo-inositol in its components. This inositol is an important constituent of the phosphatidyl-inositol, which forms part of the sperm membrane phospholipids. Accordingly, Donoghue and Wishart (Donoghue and Wishart, 2000) reported fertility levels comparable to the inseminated fresh chicken semen stored for up to 24 h at refrigeration temperatures. Reduced fecundity of stored spermatozoa may be due to the detrimental effects of damaged sperm cells (Sexton, 1988).

The poor fertilization rates obtained for avians as opposed to mammalian species are attributable to the unique morphological characteristics of the avian spermatozoa such as their filiform shape, long tail and condensed nucleus, making them more susceptible to freezing damage (Donoghue and

Wishart, 2000). Despite these, a variety of semen cryopreservation protocols involving different cryoprotective agents (CPAs), packaging methods, and freezing and thawing rates, have been developed, firstly in chicken and then in other domesticated birds, such as turkey, duck and goose (Blesbois, 2007; Donoghue and Wishart, 2000; Lake, 1986). However, previous studies indicated that the freezing process decreases the ability of sperm to acrosome-react and also decreases sperm motility (Mocé, Grasseau and Blesbois, 2010). High sperm motility rate after preservation was referred to as a key point for the high success of fertility but there were limited chicken AI studies on the effects of freezing methods on the motility of frozen-thawed sperm. Despite this, the percentage of spermatozoa shown to survive freezing and thawing is remarkably similar in most cases, at around 40–50% (Surai and Wishart, 1996). Hence, the motility of frozen-thawed semen obtained in our study, which was 58%, is acceptable for further fertility studies. It was related to other results of frozen semen in Thai-native chicken (Thananurak et al., 2017; Thananurak, et al., 2016; Thananurak et al., 2015; ).

Also, the kinetic and motion parameters of sperm is correlated with freezability and fertilizing ability. From our data, kinetic parameters of fresh semen like VCL, VSL, VAP, DCL, DSL, DAP, and ALH, BCF, and HAC were significantly higher than those of frozen-thawed semen. In contrast, values from some kinetic parameters such as WOB, LIN, and STR had no significant difference between fresh and frozen-thawed semen. Different from our results, Casas et al. (Casas et al., 2009) showed that the LIN and STR parameters were useful in predicting potential freezability of the boar sperm. However, among sperm kinetic parameters, only DCL showed an individual significant difference between fresh and frozen-thawed chicken semen. There were no reports on the correlation between sperm kinetic parameters and signaling pathways related to sperm freezability of chicken, hence, further studies should be considered.

In conclusion, this study provides a basic knowledge of Pradu-hangdam sperm characteristics and their longevity. Acceptable data was obtained even at 24 h post-storage and freezing in the BHSV extender. Moreover, fertility studies should be a future area of research towards the long-term preservation of Pradu-hangdam genetics.

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## AUTHORS CONTRIBUTION

Conceived and designed the experiments: AS

Sample collection: CK

Performed the laboratory analysis: CK, SP, AS

Analyzed the data: CK, PP

Contributed reagents/materials/analysis tools: SP, AS

Wrote the paper (review and editing): CK, SC, YZ, AS

## REFERENCES

- Abu Md Mamun, T., Bhuiyan, M., Ferdousy, R., Juyena, N., Mollah, M. B. R. (2013). Evaluation of semen quality among four chicken lines. *J. Agric. Vet.* 6, 7-13.
- Almahdi, A.B., Ondho, Y., Sutopo S. (2014). Comparative studies of semen quality on different breed of chicken in poultry breeding center Temanggung-central Java Almahdi. *Int. Ref. J. Engineer and Sci.* 3: 94- 103.
- Bakst, M. R., Sexton, T. J. (1979). Fertilizing capacity and ultrastructure of fowl and turkey spermatozoa before and after freezing. *J. Reprod. Fertil.* 55(1), 1-7.
- Blanco, J. M., Wildt, D. E., Höfle, U., Voelker, W., Donoghue, A. M. (2009). Implementing artificial insemination as an effective tool for ex situ conservation of endangered avian species. *Theriogenology*, 71(1), 200-213.
- Blesbois, E. (2007). Current status in avian semen cryopreservation. *Worlds Poult. Sci. J.* 63, 213-222.
- Blesbois, E., Grasseau, I., Seigneurin, F. (2005). Membrane fluidity and the ability of domestic bird spermatozoa to survive cryopreservation. *Reproduction*, 129(3), 371-378.
- Burrows, W. H., Quinn, J. P. (1937). The collection of spermatozoa from the domestic fowl and turkey. *Poult. Sci.* 16(1), 19-24.
- Casas, I., Sancho, S., Briz, M., Pinart, E., Bussalleu, E., Yeste, M., Bonet, S. (2009). Freezability prediction of boar ejaculates assessed by functional sperm parameters and sperm proteins. *Theriogenology*, 72(7), 930-948.
- Çiftci, H., Aygun, A. (2018). Poultry semen cryopreservation technologies. *Worlds Poult. Sci. J.* 74, 1-11.
- Donoghue, A. M., Wishart, G. J. (2000). Storage of poultry semen. *Anim. Reprod. Sci.* 62(1), 213-232.
- Feyisa, S. G., Park, Y. H., Kim, Y. M., Lee, B. R., Jung, K. M., Choi, S. B., Cho, C.Y., Han, J. Y. (2018). Morphological defects of sperm and their association with motility, fertility, and hatchability in four Korean native chicken breeds. *Asian-Australas. J. Anim. Sci.* 31(8), 1160-1168.
- Gee, G., Bertschinger, H., Donoghue, A., Blanco, J., Soley, J. (2004). Reproduction in nondomestic birds: physiology, semen collection, artificial insemination and cryopreservation. *Avian Poult. Bio. Rev.* 15, 47-101.
- Giesen, A. F., Sexton, T. J. (1983). Beltsville poultry semen extender. 9. Effect of storage temperature on turkey semen held eighteen hours. *Poult. Sci.* 62(7), 1305-1311.
- Kammongkun, J., Leotaragul, A. (2015). Estimation of genetic parameters for economic traits in Thai native chicken (Pradu-Handum Chiangmai) for fourteen generations of selection. *Khon Kaen Agr. J.* 43, 196-199. (in Thai)
- Lake, P. (1995). Historical perspective of artificial insemination technology. In: Bakst, M.R., Wishart, G.J. Eds., *Proc. 1st International Symposium on the Artificial Insemination of Poultry*. Poultry Science Association, Savoy, IL, pp. 1–20.
- Lake, P. E. (1986). The history and future of the cryopreservation of avian germ plasm. *Poult. Sci.* 65(1), 1-15.
- Long, J. (2006). Avian semen cryopreservation: What are the biological challenges? *Poult. Sci.* 85, 232-236.
- Long, J. A., Purdy, P. H., Zuidberg, K., Hiemstra, S.-J., Velleman, S. G., Woelders, H. (2014). Cryopreservation of turkey semen: Effect of breeding line and freezing method on post-thaw sperm quality, fertilization, and hatching. *Cryobiology*. 68(3), 371-378.
- Mitchell, R., Buckland, R., Kennedy, B. (1977). Heritability of fertility of frozen and fresh chicken semen and the relationship between the fertility of frozen and fresh semen. *Poult. Sci.* 56, 1168-1177.
- Mocé, E., Grasseau, I., Blesbois, E. (2010). Cryoprotectant and freezing-process alter the ability of chicken sperm to acrosome react. *Anim. Reprod. Sci.* 122(3-4), 359-366.
- Mohan, J., Sharma, S. K., Kolluri, G., Dhama, K. (2018). History of artificial insemination in poultry, its components and significance. *Worlds Poult. Sci. J.* 74, 1-14.
- Sawasdee, A.L.P., J. Kammongkun. (2015). Reproductive performance of Thai native chicken (Pradu – Handum Chiangmai) in local condition. *Khon Kaen Agr. J.* 43, 234-237. (in Thai)
- Seigneurin, F., Grasseau, I., Chapuis, H., Blesbois, E. (2013). An efficient method of guinea fowl sperm cryopreservation. *Poult. Sci.* 92(11), 2988-2996.

- Sexton, T. J. (1988). Comparison of commercial diluents for holding turkey semen 24 hours at 5 C. *Poult. Sci.* 67(1), 131-134.
- Surai, P. F., Wishart, G. J. (1996). Poultry artificial insemination technology in the countries of the former USSR. *Worlds Poult. Sci. J.* 52(1), 27-43.
- Thananurak, P., Chuaychu-noo, N., Vongpralub, T. (2017). Freezability and fertility of Thai native chicken semen in different diluents. *Thai J. Vet. Med.* 47(4), 551-556.
- Thananurak, P., Vongpralub, T., Sittikasamkit, C., Sakwiwatkul, K. (2016). Optimization of trehalose concentration in semen freezing extender in Thai native chicken semen. *Thai J. Vet. Med.* 46, 287-294.
- Thananurak, P., Sittikasamkit, C., Vongpralub, T., Sakwiwatkul, K. (2015). Effects of addition of reduced glutathione to thawing media on motility parameters, lipid peroxidation and fertility rate in frozen-thawed chicken spermatozoa. *Khon Kaen Agr J.* 43: 98-102.

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