Original Article

Comparison of sperm freezability and fertility among Hmong Black-Bone, Barred Plymouth Rock and Thai Native (Pradu Hang Dam) chickens

Saennamphung Somtaw[1] Pornjit Sonseeda[3] Nalinee Tubtimtong[1]

Yupin Phasuk[1,2] Thevin Vongpralub[1,2*]

Abstract

Semen cryopreservation is the most useful method for the long-term storage of chicken genetic resources. The objective of this study was to compare post-thaw semen quality of Hmong Black-Bone to Barred Plymouth Rock and Thai native (Pradu Hang Dam) chickens in terms of sperm motility, viability and fertility. A total of 9 males of each breed were used; semen collected from each cock was subsequently evaluated under a microscope. Semen samples were diluted with Schramm diluent, cooled down from 20–25°C to 5°C and diluent plus DMF (dimethylformamide) was added (6% DMF of final volume when the sperm concentration was 1,200 × 10^6/mL). Semen was loaded into 0.5 mL straws, frozen at -35°C for 12 mins and at -135°C for 5 min in nitrogen vapor, and then plunged into liquid nitrogen. After thawing the straws in cold water (5°C), sperm motility characteristics were analyzed by computer-assisted sperm analysis and viability was assessed by staining with SYBR-14 and propidium iodide and observing under a fluorescent microscope (400×). Fertility tests of frozen semen were carried out by artificial insemination of layer hens. There was no significant difference in motility and viability between breeds. The fertility rates for frozen semen of Hmong Black-Bone, Barred Plymouth Rock and Thai native (Pradu Hang Dam) chickens were 74.96, 69.75 and 80.59%, respectively, which showed no significant difference between breeds ($P>0.05$). The results show that semen cryopreservation in Hmong Black-Bone chickens achieved acceptable freezing ability and fertility for use in *ex situ* conservation.

Keywords: fertility, Hmong Black-Bone chicken, semen cryopreservation

[1]*Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen, 40002, Thailand*

[2]*Research and Development Network Center for Animal Breeding (Native Chicken) Faculty of Agriculture, Khon Kaen University, Khon Kaen, 40002, Thailand*

[3]*Department of Animal Science, Faculty Agriculture and Technology, Nakhon Phanom University, Nakhon Phanom, 48000, Thailand*

***Correspondence**: vongpralub@gmail.com*

Introduction

At present, more than 13% of avian species are threatened with extinction (IUCN, 2015). For domestic chickens, 35% of breeds are currently considered to be at risk of extinction (Pym, 2013) for various reasons, mainly related to modern agriculture, commercial needs and exposure to health risks.

Indigenous chickens have long played an important role in smallholder farms and among local people. Black-Bone chicken, a type of indigenous chicken, reportedly has dietary health benefits that include reducing fatigue, and anxiety, stimulating the metabolism; controlling blood sugar and blood pressure, and helping to strengthen the immune system (Li et al., 2012). Hmong Black-Bone chicken is one of the varieties of Black-Bone chicken. These indigenous chickens have been raised for food and used in traditional medicine by the Hmong people, an ethnic group living in the mountainous regions of South China, Vietnam, Laos and Thailand, and, hence, are an economically important animal for these hill tribe communities. Bouahom and colleagues (2007) reported that populations of this local high land chicken breed in Laos face the risk of extinction.

In relation to biodiversity, indigenous chickens are a reservoir of genes which may be important for future use. Currently, frozen semen preservation methods are the most practical way to maintain long-term storage of poultry semen (Blesbois et al., 2007); however, the tolerance of sperm to cryopreservation varies among chicken breeds (Long, 2006; Siudzińska and Lukaszewicz, 2008b). Many studies have shown satisfactory fertility rates of cryopreserved chicken semen (Tselutin et al., 1999; Woelders et al., 2006; Thananurak et al., 2016). To our knowledge, there is limited information available on the cryopreservation of semen from Hmong Black-Bone chicken. The objective of the present study was to determine the freezability and fertility of Hmong Black-Bone frozen semen compared to Barred Plymouth Rock and Thai native (Pradu Hang Dam) chicken frozen semen cryopreserved using a simple vapor method. The result of this study allow for the optimization of semen cryopreservation of highland indigenous chicken genetic resource.

Materials and Methods

Animals and Their Management: Two-year-old males of three breeds of domestic fowl (Hmong Black-Bone, Barred Plymouth Rock and Thai Native (Pradu Hang Dam) chickens) were used in this study. The Hmong Black-Bone roosters were purchased from farmers in the high lands, 700 m above sea level, in Kek-noi sub-district, Phetchabun province, Thailand, one year before starting the experiment. Barred Plymouth Rock is an exotic breed introduced into Thailand more than 40 years ago, while Pradu Hang Dam is a Thai native chicken breed kept by small farm holders for both meat and also as fighting cocks. The Pradu Hang Dam has black feathers, beak and leg, and white skin. Each breed was represented by 9 healthy cocks. The animals were housed in individual pens at the Animal Farm, Faculty of Agriculture, Khon Kaen University. Cocks were offered commercial feed (Balance 924, Lot No. I 160728014, Batagro Agro Industry Co Ltd Thailand) 120–130 g/day and water was provided *ad libitum*.

For fertility testing, 162 commercial layer hens (Romann Brown, Batagro Agro Industry Co Ltd Thailand) were used when they were 40 weeks old and hen day production was approximately 85% at the beginning of the experiment. The animals were reared under natural environmental conditions throughout the experimental period, July to September 2016. This experiment was carried out at the Livestock Research Farm, Faculty of Agriculture, Khon Kaen University, located at 16°26′N latitude and 102°50′E longitude, Thailand. The experiment was approved by the Animal Ethics Committee (Approval No. 33/2015) of Khon Kaen University, Thailand.

Semen collection: Semen was collected two times a week throughout the study period using the dorso-abdominal massage technique (Burrows and Quinn 1937). Care was taken to avoid contamination with cloaca products. Semen samples were collected into a 1.5 mL micro tube containing 0.1 mL Schramm diluent (Schramm, 1991), composed of 0.07 g magnesium acetate, 2.85 g sodium glutamate, 0.5 g glucose, 0.25 g inositol and 0.25 g potassium acetate dissolved in 100 mL double-distilled water. To maximize semen quality and quantity, semen collection was always performed by the same person. Semen samples were transported to the laboratory for microscopic evaluation of the mass motility score, ranging from 0–5 (0 = no sperm movement; 5 = very rapid waves and whirlwinds visible, more than 90% of sperm showing forward movement). Semen samples having good motility (at least a score of 4) were used in this study.

Freezing Procedures: Semen collected from each cock was subsequently evaluated under a microscope. Semen samples were diluted with Schramm diluent (Schramm, 1991) cooled down from 20–25°C to 5°C and diluent plus DMF (dimethylformamide) was added (6% DMF of final volume when the sperm concentration was 1,200 × 10^6/mL). Semen was loaded into 0.5 mL straws, frozen at -35°C for 12 min and at -135°C for 5 min in nitrogen vapor, and then plunged into liquid nitrogen. (Vongpralub et al., 2011) Before frozen semen assessment, straws were thawed in an iced water bath at 2–5°C for 5 mins. Three frozen semen straws from each cock were used for post-thaw motility characteristics evaluation. Sperm motility (total motile and progressive motile) was determined using a computer-assisted semen analyzer (CASA) (IVOS 12.3D; Hamilton Thorne, Beverly, MA, USA). Briefly, 5 μL samples of post-thaw semen and diluent at a ratio of 1:15 were loaded on close cover dual slides for sperm analysis. The temperature of the sample influences sperm motility (Iguer-ouada and Verstegen, 2001) since spermatozoa move more slowly at lower temperature. The optimal temperature for analysis of sperm motion is body temperature, 37–38°C (Verstegen et al., 2002). A customized CASA system was assembled, with a microscope fitted with a warming stage, negative phase-contrast optics and video camera interfaced with a computer to digitize and analyze the images (Lange-Consiglio et al., 2013). For each sample, multiple microscope fields were analyzed. Some parameters were measured directly on the digital images while others were calculated from measurements of the percentage of motile and progressive motile cells.

The percentage of sperm viability was assessed by staining with SYBR-14 and propidium iodide (PI). Since SYBR-14, a green-fluorescent cell membrane counterstain, does not penetrate dead cells, it is commonly used to identify live cells in a population; while PI, a red-fluorescent nuclear and chromosome counterstain, does not penetrate live cells and is commonly used to identify dead cells in a population. The protocol of two types of fluorescent staining was as follows: 2 μL SYBR-14 was added to 150 μL post-thaw semen and incubated for 10 min; next, 2 μL PI was added and incubated for 5 min; then samples were evaluated under a fluorescent microscope (400×) (Olympus 1X71, Tokyo, Japan), as described by Partyka et al. (2010). For sperm viability assessment, three frozen semen straws from each male were used.

Artificial Insemination: Assessment of the fertilizing ability of frozen semen was carried out by single intra-vaginal insemination of layer hens. A total of 162 hens were divided into 27 groups of 6 hens, and each group was assigned to be inseminated once by frozen semen pooled from six ejaculates of each cock. Artificial insemination was performed using a tuberculin syringe containing 0.4 mL of frozen semen (the insemination dose was about 480 million spermatozoa/artificial insemination) inserted into the vagina (about 4 cm). The fertilizing ability of sperm cells was estimated from eggs collected on day 2–8 post-insemination. Fertility rates (fertilized eggs / incubated eggs × 100) were determined by candling eggs on day 7 of incubation.

Statistical Analysis: The percentages of sperm motility, viability and fertility were examined by randomized complete block design; statistical analysis of data used the generalized linear model procedure of SAS (1996). The means of the treatment effects were compared using Duncan's multiple range test (P<0.05).

Results

Fresh Semen Parameters: Fresh semen quality was determined from the pooled ejaculates of each breed, six times per breed, during the collection period. Mean semen volume, motility score (0–5) and sperm concentration for Hmong Back-Bone, Barred Plymouth Rock and Thai native (Pradu Hang Dam) chickens, respectively, were: 0.311.11 ± 24.84 mL, 4.68 ± 0.05 and 3,054.63 ± 108.34 million cells/mL; 0.366.67 ± 25.15 mL, 4.73 ± 0.02 and 3,112.96 ± 143.44 million cells/mL; and 0.320.37 ± 22.86 mL, 4.70 ± 0.03 and 3,199.07 ± 171.92 million cells/mL (data not shown in tables).

Post-Thaw Sperm Characteristics: Mean total motility and progressive motility parameters of thawed spermatozoa are shown in Table 1. There were no differences among breeds (P>0.05). There also was no statistically significant difference (P>0.05) in the percentage of sperm viability of thawed sperm from Hmong Black-Bone, Barred Plymouth Rock and Thai native (Pradu Hang Dam) chickens. Significant (P<0.05) differences between individual males in each breed were found for all post-thawed sperm parameters.

Fertility of Cryopreserved Semen: The effect of breed on fertility of frozen semen is shown in Table 2. There were no statistically significant differences (P>0.05) among breeds in fertility. Significant (P<0.05) differences were found within breeds regarding fertility.

Discussion

The present study was undertaken to investigate the sperm freezing ability and fertility of Hmong Black-Bone chickens compared to Barred Plymouth Rock and Thai native (Pradu Hang Dam) chickens, under a suitable protocol for chicken semen cryopreservation which could pave the way for *ex situ* conservation of this highland chicken.

It is generally accepted that the ability of spermatozoa to survive under cryopreservation is different in different avian species (Blanco et al., 2008; Blanco et al., 2012) and in different breeds or strains (Hammerstedt, 1995; Siudzińska and Lukaszewicz, 2008a; Makhafola et al., 2009; Purdy et al., 2009; Long et al., 2010); intra-species variations in freezability have also been reported (Blesbois et al., 2007; Kowalczyk and Lukaszewicz, 2015).

Massip and colleagues (2004) reported that the quality of frozen semen of chickens and ganders was superior to most other poultry. A study by Blesbois and colleagues (2005) also found that post-thawed fowl semen showed significantly higher percentages of viable and morphologically normal spermatozoa compared to the semen of turkeys and guinea fowl. Numerous studies have reported on the effect of breed on the freezability of domestic fowl semen. In relation to freezability, Siudzińska and Lukaszewicz (2008a) found that post-thawed semen quality was significantly different among four fancy fowl breeds; this was in agreement with a previous study by Tselutin and colleagues (1995), in which the cryopreserved semen of four fancy fowl breeds was assessed. Peters and colleagues (2008) compared the quality of frozen semen of 7 chicken breeds and found differences in post-thawed semen quality, although the same processing method was used. Similarly, Blesbois and colleagues (2007) and Wishart (2009) reported that the quality of frozen semen depends on differences between breeds. Long and colleagues (2010) also reported that there was significant variability in the frozen semen quality of eight poultry lines, which supported the results of previous studies.

The present experiment indicated that there was similar post-thawed semen quality of Hmong Black-Bone chickens and the two other breeds mentioned above. This is in accordance with previous reports: Mosenene (2009) showed that there were no significant differences between breeds in the frozen semen quality of Rhode Island Red, Potchefstroom Koekoek, New Hampshire and White Leghorn chickens; and Makhafola and colleagues (2009) reported that there was no difference in sperm survivability immediately after thawing between Potchefstroom Koekoek and Ovambo breeds. Recently, Sonseeda (2013) studied three Thai native chicken lines (Leung Hang Khao, Pradu Hang Dam and Chee) to determine differences in cryopreserved semen quality; the results showed that the line of Thai native cocks had no effect on post-thaw semen quality.

In the present study, a significant variation of intra-breed post-thawed semen quality was obtained (Table 1), even though good raw semen samples were used. Many biological and biophysical factors may affect the ability of sperm cells to prevent damage caused by the freezing/thawing procedure (Holt, 2000). These factors include membrane permeability, lipid composition and fluidity (which affect motility), and different metabolic factors, including ATP concentration in spermatozoa post-cryopreservation (Wishart and Palmer, 1986). The determination of sperm membrane permeability in various avian species under different osmotic conditions indicates that spermatozoa fluidity differs between species of poultry (Blesbois et al., 2005). These differences partly reflect differences in cholesterol content and may be related to species differences in semen freezability. In chickens, intra-species variations in cholesterol/phospholipid content were also found to be related to semen freezability (Ansah and Buckland, 1982). The intra-breed differences in freezability in the present study may depend on variations in the cholesterol/phospholipid content of sperm membranes in semen from individual cocks.

Table 1 Sperm freezability of Hmong Black-Bone, Barred Plymouth Rock and Thai native (Pradu Hang Dam) chickens

Breed	Male #	Motility parameters (%)							Viability
		Motile	Progressive	VCL	VSL	VAP	LIN	ALH	
Hmong Black-Bone	1	64.00^{a}	16.67^{bc}	135.07^{ab}	57.57^{abc}	77.10^{abc}	43.00^{ab}	5.87^{a}	44.27^{ab}
	2	55.67^{bc}	14.33^{bcd}	118.77^{c}	46.83^{cd}	66.47^{cd}	40.67^{d}	5.30^{ab}	41.46^{b}
	3	40.67^{bc}	14.00^{bcd}	130.53^{abc}	54.00^{abcd}	70.67^{bc}	42.00^{bc}	5.63^{ab}	44.86^{ab}
	4	62.33^{a}	14.67^{bcd}	135.37^{ab}	59.27^{ab}	78.87^{ab}	44.00^{ab}	5.63^{ab}	45.26^{ab}
	5	59.33^{ab}	19.33^{ab}	141.07^{a}	64.33^{a}	83.93^{a}	45.67^{a}	5.93^{a}	47.92^{a}
	6	50.67^{a}	13.33^{bcd}	134.27^{ab}	61.00^{ab}	78.10^{abc}	45.33^{a}	5.97^{a}	43.41^{ab}
	7	32.67^{c}	10.00^{d}	124.93^{bc}	50.63^{bcd}	69.87^{bc}	41.33^{cd}	5.67^{ab}	46.22^{ab}
	8	60.67^{ab}	23.00^{a}	106.63^{d}	43.33^{d}	56.67^{d}	42.33^{bc}	4.67^{b}	45.99^{ab}
	9	51.67^{ab}	10.67^{cd}	135.77^{ab}	54.43^{abcd}	71.57^{bc}	41.33^{cd}	5.63^{ab}	47.81^{a}
	Mean ± SE	53.07±3.51	15.11±1.36	129.16±3.57	54.60±2.77	72.58±2.69	42.85±0.60	5.59±0.13	45.24±0.69
Barred Plymouth Rock	1	54.67^{abc}	10.33^{cd}	127.83^{bc}	55.83^{ab}	74.00^{ab}	44.00^{a}	5.77^{bc}	42.69^{bc}
	2	67.67^{a}	19.67^{a}	143.77^{a}	60.43^{a}	80.53^{a}	42.67^{a}	6.13^{ab}	42.89^{bc}
	3	64.33^{ab}	16.67^{ab}	132.10^{ab}	59.87^{a}	75.87^{ab}	45.00^{a}	5.20^{c}	45.29^{b}
	4	50.67^{abc}	9.67^{b}	137.60^{ab}	60.00^{a}	79.23^{a}	43.33^{a}	6.67^{a}	40.58^{c}
	5	42.33^{bc}	10.00^{b}	113.00^{d}	49.60^{b}	63.70^{c}	45.67^{a}	5.50^{c}	50.57^{a}
	6	46.00^{abc}	11.33^{b}	138.67^{ab}	58.93^{a}	77.57^{ab}	43.00^{a}	6.47^{a}	45.42^{b}
	7	40.67^{c}	16.00^{ab}	116.93^{cd}	52.90^{ab}	69.13^{bc}	45.33^{a}	5.47^{c}	45.98^{b}
	8	67.33^{a}	10.33^{b}	138.23^{ab}	51.40^{ab}	72.00^{abc}	38.33^{b}	6.43^{a}	44.27^{bc}
	9	59.33^{abc}	15.00^{ab}	126.07^{bc}	52.90^{ab}	68.80^{bc}	43.00^{a}	5.23^{c}	51.78^{a}
	Mean ± SE	54.78±3.50	22.00±1.22	130.47±3.48	55.76±1.39	73.43±1.84	43.37±0.73	5.87±0.19	45.50±1.21
Pradu Hang Dam	1	59.00^{ab}	20.67^{a}	135.83^{ab}	66.70^{ab}	84.07^{ab}	49.33^{a}	5.83^{ab}	45.35^{bc}
	2	66.00^{a}	15.67^{ab}	142.23^{ab}	58.20^{abc}	80.30^{ab}	42.00^{b}	5.93^{a}	44.27^{c}
	3	71.33^{a}	21.67^{a}	143.97^{a}	67.70^{a}	86.50^{a}	47.00^{ab}	6.10^{a}	40.42^{d}
	4	64.33^{a}	18.33^{a}	144.00^{a}	63.73^{abc}	83.83^{ab}	44.33^{ab}	6.07^{a}	46.17^{bc}
	5	48.00^{b}	14.00^{ab}	127.93^{ab}	55.73^{bc}	72.93^{b}	44.00^{ab}	5.63^{ab}	39.91^{d}
	6	58.67^{ab}	15.33^{ab}	136.70^{ab}	58.17^{abc}	75.93^{ab}	42.33^{b}	5.50^{ab}	48.57^{ab}
	7	57.67^{ab}	19.33^{a}	126.50^{b}	58.07^{abc}	72.87^{b}	46.67^{ab}	5.03^{b}	50.19^{a}
	8	65.67^{a}	22.00^{a}	132.50^{ab}	60.97^{abc}	77.40^{ab}	46.33^{ab}	5.63^{ab}	48.94^{ab}
	9	30.00^{c}	14.33^{b}	126.87^{b}	54.13^{c}	72.30^{b}	44.67^{ab}	5.30^{ab}	51.83^{a}
	Mean ± SE	57.85±4.13	17.37±1.40	135.17±2.39	60.38±1.58	78.46±1.81	45.18±0.79	5.67±0.12	46.18±1.39

Different superscripts within a column indicate significant differences (P<0.05)

The results of this study indicated that the fertility ranges were 58.54–92.31% for Hmong Black-Bone, 46.34–87.5% for Barred Plymouth Rock and 41.18–100% for Thai native (Pradu Hang Dam) chickens, respectively; high intra-breed variation and significant differences (P<0.05) among cocks within breed were obtained. These results are contradictory to previous findings, where the fertility of frozen semen of inbred chickens was different among groups (Bacon et al., 1986; Kirby et al., 1998). Blesbois and colleagues (2005) also reported that post-thawed native chicken semen had lower fertility compared with commercial breed chickens. In poultry, genetic selection for sperm freezability has the ability to improve the frozen semen quality of chickens (Siudzińska and Lukaszewicz, 2008; Long et al., 2010), geese (Lukaszewicz, 2010) and turkeys (Iaffaldano et al., 2008; Long et al., 2014). This suggests that random breeding conserved biodiversity and resulted in a variation of fertility rates of cryopreserved semen. We propose that in the present study the high individual within- breed variation caused non- significant differences in fertility among breeds. This result is in accordance with a previous report by Hammerstedt (1995), who showed that the range of frozen semen fertility in chickens was 0–90% (mean fertility 60%), and also the results of Blesbois and colleagues (2008), which showed that the fertility rate of chicken frozen semen was from 9 to 94%, a wider range compared with fresh semen (34–94%). The high variation in cryopreserved semen fertility of individual chickens may be related to many biological and biophysical factors, including a loss of sterols from membranes and a depletion of membrane rafts (Ushiyama et al., 2016), that affect individual differences in semen freezability.

In summary, fertility rates were not significantly different among Hmong Black-Bone, Barred Plymouth Rock and Thai native (Pradu Hang Dam) chicken breeds. The present study showed high individual intra-breed variation in post-thawed semen quality and fertility. The fertility rates, obtained after one-time artificial insemination with frozen semen, of all breeds were superior to the fertility standard (60%) of chicken cryopreserved semen. The fertility results of 74.96% for Hmong Black-Bone chicken cryopreserved semen, achieved using a simple vapor freezing procedure, suggest the feasibility of establishing a sperm bank for the conservation of Hmong Black-Bone genetic resources.

Table 2 Fertility rates of frozen semen of Hmong Black-Bone, Barred Plymouth Rock and Thai native (Pradu Hang Dam) chickens

Breed	Male #	Number of eggs	Fertility (%)
Hmong Black-Bone	1	39	84.62[ab]
	2	42	73.81[bc]
	3	40	65.00[cd]
	4	35	68.57[cd]
	5	38	92.11[a]
	6	39	92.31[a]
	7	41	58.54[d]
	8	42	71.43[bc]
	9	41	65.85[cd]
	Mean ± SE		74.96±4.03
Barred Plymouth Rock	1	38	52.63[bc]
	2	41	75.61[ab]
	3	37	72.97[ab]
	4	42	83.33[a]
	5	40	65.00[bc]
	6	41	46.34[d]
	7	40	87.50[a]
	8	42	73.81[ab]
	9	38	71.05[ab]
	Mean ± SE		69.75±4.48
Pradu Hang Dam	1	34	41.18[d]
	2	40	100.00[a]
	3	39	64.10[bc]
	4	42	95.23[a]
	5	41	85.37[ab]
	6	40	100.00[a]
	7	42	85.71[ab]
	8	37	81.08[ab]
	9	41	68.29[bc]
	Mean ± SE		80.59±6.44

Different superscripts within a column indicate significant differences ($P<0.05$)

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บทคัดย่อ

การศึกษาเปรียบเทียบความทนได้ในการแช่แข็งและอัตราการผสมติดในไก่กระดูกดำสายพันธุ์ม้ง ไก่บาร์พลีมัทร็อค และไก่พื้นเมืองไทย (ประดู่หางดำ)

แสนน้ำผึ้ง สมท้าว[1] พรจิต สอนสีดา[3] นลินี ทับทิมทอง[1] ยุพิน ผาสุข[1,2] เทวินทร์ วงษ์พระลับ[1,2]*

การเก็บรักษาน้ำเชื้อด้วยวิธีการแช่แข็งเป็นวิธีการที่เป็นประโยชน์ที่สุดในการเก็บรักษาทรัพยากรพันธุกรรมของไก่ การศึกษานี้มีวัตถุประสงค์เพื่อ เปรียบเทียบคุณภาพน้ำเชื้อภายหลังการทำละลายของไก่กระดูกดำสายพันธุ์ม้ง กับไก่บาร์พลีมัทร็อค และไก่พื้นเมืองไทย (ประดู่หางดำ) ในด้านอัตราการเคลื่อนที่ของอสุจิ อัตราการรอดชีวิต และอัตราการผสมติด การศึกษาครั้งนี้ใช้พ่อพันธุ์สายพันธุ์ละ 9 ตัว ทำการรีดเก็บน้ำเชื้อจากพ่อพันธุ์ไก่ ประเมินคุณภาพของน้ำเชื้อพ่อพันธุ์แต่ละตัวภายใต้กล้องจุลทรรศน์ เจือจางตัวอย่างน้ำเชื้อด้วยน้ำยาเจือจางสูตร Schramm ลดอุณหภูมิจาก 20-25 องศาเซลเซียส ไปที่ 5 องศาเซลเซียส และเจือจางน้ำเชื้อด้วยน้ำยาเจือจางสูตรที่มี DMF (dimethyl formamide) ความเข้มข้นสุดท้าย 6 % โดยมีอสุจิที่ความเข้มข้น 1,200 ล้านตัว/ มิลลิลิตร บรรจุน้ำเชื้อลงในหลอดฟางขนาด 0.5 มิลลิลิตร แช่แข็งที่อุณหภูมิ -35 องศาเซลเซียส เป็นเวลา 12 นาที และที่ -135 องศาเซลเซียส เป็นเวลา 5 นาที ในไอไนโตรเจนเหลว จากนั้นบรรจุไว้ในถังไนโตรเจนเหลว ทำละลายน้ำเชื้อในน้ำเย็น (5 องศาเซลเซียส) ทำการตรวจวิเคราะห์อัตราการเคลื่อนที่ของอสุจิโดยเครื่อง computer-assisted sperm analysis (CASA) และตรวจวิเคราะห์อัตราการรอดชีวิตโดยการย้อมสี SYBR-14 and propidium iodide ตรวจประเมินภายใต้กล้องกำลังขยาย 400 เท่า ทดสอบอัตราการผสมติดโดยการผสมเทียมในแม่พันธุ์ไก่ไข่ ผลการศึกษาพบว่าอัตราการเคลื่อนที่และอัตราการรอดชีวิตของอสุจิระหว่างไก่แต่ละสายพันธุ์ ไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติ อัตราการผสมติดของน้ำเชื้อแบบแช่แข็งของไก่กระดูกดำสายพันธุ์ม้ง ไก่บาร์พลีมัทร็อค และไก่พื้นเมือง (ประดู่หางดำ) มีค่าเท่ากับ 74.96, 69.75 และ 80.59 เปอร์เซ็นต์ ตามลำดับ ซึ่งไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติระหว่างสายพันธุ์ ($P>0.05$) ผลการศึกษาชี้ให้เห็นว่าน้ำเชื้อแบบแช่แข็งของไก่กระดูกดำสายพันธุ์ม้งมีความทนได้ต่อการแช่แข็ง และอัตราการผสมติดสูงในระดับมาตรฐาน น้ำเชื้อแบบแช่แข็ง จึงสามารถใช้เป็นเครื่องมือในการอนุรักษ์พันธุกรรมของไก่กระดูกดำสายพันธุ์ม้งได้

คำสำคัญ: อัตราการผสมติด ไก่กระดูกดำสายพันธุ์ม้ง น้ำเชื้อแช่แข็ง

[1]*ภาควิชาสัตวศาสตร์ คณะเกษตรศาสตร์ มหาวิทยาลัยขอนแก่น ขอนแก่น ประเทศไทย 40002*

[2]*ศูนย์เครือข่ายวิจัยและพัฒนาด้านการปรับปรุงพันธุ์สัตว์ (ไก่พื้นเมือง) คณะเกษตรศาสตร์ มหาวิทยาลัยขอนแก่น ขอนแก่น ประเทศไทย 40002*

[3]*สาขาวิชาสัตวศาสตร์ คณะเกษตรและเทคโนโลยี มหาวิทยาลัยนครพนม นครพนม ประเทศไทย 48000*

***ผู้รับผิดชอบบทความ** E-mail: vongpralub@gmail.com*