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

Effect of extenders on frozen semen quality of Thai native chicken (Lueng hang kao)

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ABSTRACT: This present study examined the feasibility of cryopreservation of Thai native chicken "Luang hang kao" spermatozoa. Effects of eight extenders (modified extenders with osmotic pressure of 300, 350 and 400 mOsmol/kg, Lake's diluent, BPSE, IGGKP, EK and Schramm) with the use of dimethyl formamide (DMF 6%) as cryoprotectant on the cryopreservation of Thai native chicken (Lueng hang kao) sperm were investigated. The highest motility, progressive motility and viability percentages were resulting from modified extender with an osmotic pressure of 350 mOsmol/kg, EK and Schramm ($P<0.05$).
Keywords: Extender, Cryoprotectant, Frozen semen, Native chicken

Introduction

Thai native chickens are still an economic important to rural community in Thailand. They serve as a major source of protein for home consumption. Outbreaks of avian influenza in 2003 caused drastic losses in numbers of their breed line in the genetic of native chickens. In addition, the chicken ejaculates a limited volume of semen which is one of the constraints for artificial insemination. Chicken semen has a high concentration of about 6 to 12 million sperm/mL (Donoghue and Wishart, 2000). Assisted reproductive technologies such as artificial insemination and semen cryopreservation play an important role in preserving and transfusing valuable genes to the future (Sandanand et al., 2004). One of the most critical steps in successful cryopreservation of fowl semen is the choice of the extender. There are basic characteristics common to nearly all diluents: factor to maintain pH, osmolality and provide an energy source for spermatozoa (Christensen, 1995). The advantages of using one extender over the others are not clear, although most standard extenders seem to work reasonably well across similar species, but there is a lack of information on suitable extenders for the cryopreservation of Thai native chicken spermatozoa. There is a need to develop a suitable extender for diluting and preserving chicken spermatozoa.

Material and Methods

Animals and Semen collection

The experiment was conducted on 30 mature native cocks (Leung hang kao) kept in individual cages under natural light temperature conditions at the Suranaree University of Technology Farm, Nakhon Ratchasima, Thailand. Cocks were fed 110 g/day/bird and water was provided *ad libitum*. Semen was collected from each male twice a week by dorso-abdominal massage following the Bur-

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rows and Quinn method (1937) and the sperm were pooled and used for cryopreservation.

Semen cryopreservation

The pooled sperm was diluted with each extender (Modified extenders with osmotic pressure of 300, 350 and 400 mOsmol/kg. (Boonmatan, 2014), Lake's diluent (Lake, 1968), Beltsville Poultry Semen (BPSE) (Sexton, 1977), IGGKP (Lukaszewicz, 2001), EK (Lukaszewicz, 2002) and Schramm (Chalah, 1999) at a ratio of 1: 2 (sperm: extender). The composition of each extender is shown in Table 1. The diluted sperm was cooled down to 5 ºC and mixed with 6% DMF. Semen was loaded into 0.5 mL French straws. In freezing, liquid nitrogen was poured into styrofoam box to a level of 8 cm, then the straws were placed at 11 cm and 3 cm heights from the liquid nitrogen surface for 12 min and 5 min, respectively. The frozen sperm samples were plunged into liquid nitrogen.

Measurement of sperm motility and viability

For each assessment of motility, the frozen sperm was diluted in each extender with a ratio of 1:15 and dropped onto 2X-CEL slide sperm analysis chamber and covered with coverslip and motility analysis started thereafter. Motility of sperm samples was determined using computer assisted sperm analysis (CASA; IVOS; Hamilton-Thorne, USA). CASA settings were optimized to ensure a good detection of chicken spermatozoa (frame rate = 60 Hz, cell size = 4 pixels, minimum cell size = 4 pixels, cell intensity = 50, minimum contrast = 25, straightness (STR) threshold = 80 %, VAP cutoff = 5 μm/sec, progressive min VAP = 20 μm/sec, VSL cutoff = 20 μm/sec and temperature = 37˚C). Motility parameters assessed with CASA were total motility (%) and progressive motility (%). Sperm viability was determined using an eosin-nigrosin dry. The viability procedure was similar to that described by Kwantong (2003).

Statistical analysis

All data were expressed as means ± standard error. The motility and viability percentages were subjected to arcsine transformed prior to analyze. Data were analyzed by one-way analysis of variance (ANOVA) with subsequent of Duncan's new multiple range test, at a probability level of $P<0.05$.

Results and Discussion

An evaluation of the extenders on quality of cryopreserved Thai native chicken (Leung hang kao) semen showed that modified extenders with an osmotic pressure of 350 mOsmol/kg, EK and Schramm yielded the higher motility percentage (46.67±2.85, 43.67±2.33 and 46.00±3.06, respectively), progressive motility percentage (22.33±2.40, 21.67±0.67 and 22.00±3.06, respectively) and viability percentage (50.00±1.73, 51.00±0.58 and 46.67±2.40, respectively) than that of the other treatments ($P<0.05$; **Figure 1**). Thai native chicken (Leung hang kao) semen has an osmolality and pH of seminal plasma was 340.00±0.58 mOsmol/kg and 7.31±0.01, respectively. Hyperosmolarity (414±0.67 mOsmol/kg) of the EK extender and Schramm extender (415±0.33 mOsmol/kg) in this study did not cause adverse morphological changes in sperm morphology. These results were similar to Lukaszewicz et al. (2004), who reported EK as extender yielded the good results in gander semen. For the modified extenders which fructose were added at the con-

centration of 0.1976, 1.648 and 2.1568 % for Mo 300,Mo 350 and Mo 400 groups respectively, this make it different on osmotic pressure, the Mo 350 was superior to Mo300 and Mo 400 groups. This result supported by the report of Christensen (1995) in which the optimal osmolarity of extender for chicken semen was 325-350 mOsmol/kg. For the other groups, the standard of poultry extenders, there are difference in composition among extenders which lead to the different of their properties including pH and osmotic pressure. It is unclear that Lake, a well-known standard extender resulted lower post-thaw quality of semen. Christensen(1995) has suggested that there are many poultry extender and should be used under its recommend ideal condition. In the present study, cryopreservation procedure was carried out by simple vapor method which may be suitable for some not all extender. Base on the results of this study, It might be concluded that our Modified extender with an osmotic pressure of 350 mOsmol/kg, EK and Schramm are more effective on motility and viability of cryopreserved Thai native chicken (Leung hang kao) semen. Moreover, further studies need to ascertain the effect of different extender on the fertilization ability in cryopreserved Thai native chicken (Leung hang kao) semen.

Table 1 Composition of eight extenders used for cryopreservation of Thai native chicken (Leung hang kao) semen.

Composition	Dilutent (g/50ml)							
	Mo 300*	Mo 350**	Mo 400***	Lake's	BPSE	IGGKP	EK	Schramm
Magnesium acetate	0.0400	0.0400	0.0400	0.0584	0.0170	-	-	0.0350
Sodium acetate	-	-	-	-	0.2150	-	-	-
Potassium citrate	0.2000	0.2000	0.2000	-	0.0340	0.0700	0.0741	-
Sodium glutamate	1.0000	1.0000	1.0000	1.0622	0.4884	0.7000	0.7000	1.4250
Dipotassium hydrogen phosphate	-	-	-	-	0.8320	0.4900	-	-
Potassium dihydrogen phosphate	-	-	-	-	0.0326	-	-	
Fructose	0.0988	0.5324	1.0784	0.4000	-	-	0.1000	-
TES****	1.0000	1.0000	1.0000	-	0.0976	-	-	-
Potassium acetate	-	-	-	0.2960	-	-	-	0.2500
Polyvinylpyroridone (PVP)	-	-	-	0.1500	-	-	0.0500	-
Glucose	-	-	-	-	0.2500	0.4500	0.3500	0.2500
Sodium dihydrogen phosphate	-	-	-	-	-	0.1050	0.1050	-
Inositol	-	-	-	-	-	0.4500	0.3500	0.1250
Protamine sulfate	-	-	-	-	-	-	0.0100	-
Anhydrous sodium hydrogen phosphate	-	-	-	-	-	-	0.4900	-
Osmolarity (mOsmol/kg)	303±1.73	353±2.00	404±1.73	370±0.67	394±0.67	369±0.33	414±0.67	415±0.33
pH	7.44±0.02	7.45±0.01	7.42±0.03	7.04±0.01	7.65±0.03	7.25±0.01	7.26±0.02	6.96±0.01

*, **, *** Modified extenders with osmotic pressure of 300, 350 and 400 mOsmol/kg, respectively.

**** (N-Tris (hydrxy methal) Methyl-2-amino ethane sulfonic acid

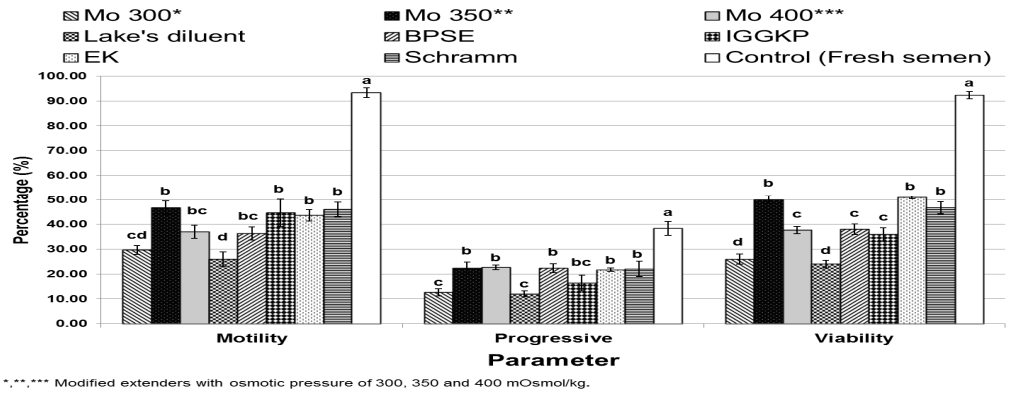

Figure 1 Effect of extenders on frozen semen quality of Thai native chicken (Lueng hang kao). Different letters indicate significant different (P<0.05)

Acknowledgements

This study was supported by the Suranaree University of Technology. The authors would like to thank to Suranaree university farm for support the Thai native cocks (Leung hang kao) and the animal care staff at the Suranaree university farm.

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