OPEN ACCESS International Journal of Applied Biology

ISSN : 2580-2410
eISSN : 2580-2119

Sperm Fertility of SK Kedu Chicken in Lactated Ringer's-Egg Yolk Extender with 10% of DMSO

Sipora Petronela Telnoni [1]*, R. Iis Arifiantini [2], Sri Darwati [3]

[1] Faculty of Mathematics and Science, San Pedro University, Kupang, East Nusa Tenggara.
[2] Faculty of Veterinary Medicine, IPB University, Bogor, West.
[3] Faculty of Animal Science, IPB University, Bogor, West Java.

Abstract

This study was conducted to evaluate fertility of SK Kedu chicken post inseminated by frozen semen using lactated ringer's-egg yolk with 10% of DMSO. Frozen semen of lactated ringer's-egg yolk with 10% of DMSO was picked from container of Liquid Nitrogen (-196 ºC) and thawed in warm water at 37 ºC for 30 second. Frozen semen inseminated to 3 arab hens: A1, A2 and A3 with 100 motile sperm cell in 0.25 mL-1 using 2 straws and deposited into reproductive tract of hen as long as 7 cm in intra utein using AI gun. Egg was collected in day 2 after AI till day 14, labelled and incubated. Sperm fertility was estimated by using % sperm fertility formulation = fertile eggs / incubated eggs x 100, regardless of whether the eggs will hatch or not. Egg fertility are checked on 5 day after incubation. This result showed % sperm fertility of SK kedu chicken were A1 (77.78 %), A2 (33.33 %), and A3 (83.33 %). The mean of % sperm fertility was 72.22 %. It can be concluded that lactated ringer's-egg yolk with 10% of DMSO can maintain sperm fertility of SK kedu chicken.

Article History
Received 26 October 2020
Accepted 03 July 2021

Keyword
Lactated ringer's-Egg yolk, DMSO, Sperm Fertility, Artificial Insemination, Chicken.

Introduction

Sperm fertility for chicken is a manifestation of the fertilization process of spermatozoa in the female reproductive tract. Best manifestation of fertilization gives contribution for chicken breeding value and to maintain the population. Further more, artificial insemination (AI) as a tool of biotechnology which use for breeding in economic purpose and increase population of chicken widely used require ability of sperm fertility. The prediction of sperm fertilizing ability has great economic importance for breeding herds when artificial insemination is used (Gadea, 2005). SK kedu chicken from Indonesia biodiversity has the good ability for maintaning economic purpose by using AI. SK kedu is a the one of superior cross-breed chickens. SK kedu chicken is a chicken came from the cross breed of 3 Indonesia local chickens i.e sentul chicken, kampung chicken, and kedu chicken. 3 local chicken in SK kedu have the characteristics: Sentul chicken has advantages, that is as a producer of meat and eggs (dual use type), male Sentul chicken body weight 1.3 kg to 3.5 kg and female chickens 0.8 kg to 2.2 kg, egg production 118 eggs per year (Diwyanto *et al*., 2011), kampung chicken can weigh 1 815 g (male) and 1 382 g (female) (Mulyono &

Pangestu, 1996). The production of native chicken eggs is 80 eggs per parent per year (Sinurat *et al*., 1992) and kedu chicken can weigh 2.0 kg to 4.0 kg. Kedu chicken has the advantage of high egg production is 124 eggs per year (Krista & Harianto, 2013).

SK kedu as cross-breed Indonesia native chicken have the potential in breeding. This potential can be developed as breeding using AI. Implementation of AI using frozen semen in cryopreservation is an important because it is able to store genetic material of SK kedu chickens which have advantages and a limited number of breeds. The frozen semen is a result of cryopreservation which using to maintance genetic of some rare spesies preserve cell, included by reproductive cell of poultry. Poultry semen cryopreservation is considered to be a challenging method to preserve reproductive cells (Blesbois, 2011; Kowalczyk & Łukaszewicz, 2015).

Cryopreservation of chicken semen requires a number of supporting factors, including the type of diluent, cryoprotectant and concentration of cryoprotectant. These supporting factors will affect the quality of frozen semen. The criteria for the quality of frozen semen that were assessed included of % sperm motility after thawing and % sperm fertility after AI. The ability of sperm fertility is also determined by buffer system, nature of cryoprotectants and additives such as sugars, calcium chelators, antioxidants and milk or egg yolk proteins (Holt, 2000).

Sperm fertility from frozen semen in cryopreservation methods has difference value of sperm motility that indicated % ability of sperm to fertilise ovum. The assessment sperm motility is influenced by the structure and integrity of the sperm function and allows prediction of the success of sperm fertility (Froman, 2007; Blesbois *et al*., 2008). The positive result of fertility in chickens depends on several factors including the ability of the spermatozoa to undergo acrosome reactions at fertilization. The acrosome reaction is an exocytotic process that involves membrane fusion and is required to achieve fertilization (Lemoine *et al*., 2011; Nguyen *et al*., 2014).

Lactated Ringer's and Egg Yolk have the ability to maintance frozen sperm quality included sperm fertility. The use of egg yolks has shown a good benefit in cryopreservation of semen, that is as a protector of the plasma membrane and the acrosome of spermatozoa when cold shock occurs (Amira *et al*., 2004). Lactated Ringer's contains a water source and electrolytes, produces a metabolic alkalinizing effect, and contains a number of chemical compounds, water, pH value, osmolarity, and energy source. Jones (1997) explains that the lactate is one of the energy sources that can be used to replace glucose and fructose for spermatozoa, which fructose is the major fuel for the mitochondrial production of ATP.

DMSO is a very common CPA that is used at various concentrations (Lovelock & Bishop 1959; McGann & Walterson, 1987) and is exceptionally effective for the cryopreservation of a variety of biological tissues and used universally for stabilizing cell membranes under rapidly changing conditions, preventing intracellular ice crystal formation during freezing and heat release during the period of phase transition (Hubalek, 2003; Cottler, 2009).

AI in chicken is a semen distribution method (plasma semen and sperm) to female reproductive tract. AI in chicken first reported in 1936 (Getachew, 2016). AI in chicken is based on 2 steps: 1). Semen is collected from rooster and 2). Semen inseminated to hen, with the main purpose is collecting the fertile eggs (Bakst & Dymond, 2013). Collecting semen in rooster for AI uses abdominal massage method (Bakst & Long, 2010). Massage do under the back of abdomen to cloaca for phallus exudation, further massage around cloaca's area for semen excretion. Implementation of the AI process, batch of selected

sperm will be transferred to the main site of sperm at sperm storage tubules (SST). Sperm will exit from SST and transported to infidibulum as fertilization site dan it is function as the second site sperm storage. Fertilized ovum will be transferred to magnum as albumin secretion site, and continues in to isthmus as the site of shell membrane formation and shell formation of egg till ovoposed. Chicken uses 24-26 hours for formation of follicle (first follicle) and will be ovulated (Bakst and Dymond 2013)..

Materials and Methods

Sample This study was conducted after approval by animal care and use committee of IPB University, number 028/ACUC/10/2016. 5 SK kedu roosters at 48 weeks and 3 arab hens at 30 weeks were used in this study. All roosters and hens fed with 100 g commercial diet (17% crude protein) individual 1 day -1 and water was provided ad libitum. Roosters and hens were housed in individual battery cages. Artificial insemination (AI) was done at 4-5 pm using frozen semen in AI procedur with ≥ 40 % motility post thawed and 100 cell concentration of motil sperms in 0.25 mL -1 and positioned in reproduction tract of arab hen using AI gun. AI gun positioned along 7 cm in intra uterin. Composisition of frozen semen extender showed in table 1 and table 2. Egg was collected in day 2 after AI till day 14, labelled and incubated. Sperm fertility was estimated by using % sperm fertility formulation = fertile eggs / incubated eggs x 100 (Brillard 2003).

Results and Discussion

Sperm fertility showed the ability of sperms to ovum fertilization. Based on this study, ovum of arab hens was showed it is fertilized by sperm in frozen semen sperm of SK kedu chicken in lactated ringer's-egg yolk with 10% of DMSO post AI. This result indicated that quality of sperm in frozen semen post thawed has the ability to fertilization the ovum (table 3).

% sperm fertility between 3 arab hens was showed different, A1 (1 arab hen), A2 (2 arab hen), and A3 (3 arab hen). A1 and A3 resulted % sperm fertility was higher than A2 % sperm fertility. Different result in % sperm fertility of AI caused by the best time of AI when positioned in reproduction tract of arab hen using AI gun. Holt (2000) explained that the correct insemination time is very important when using frozen semen because the duration of the survival of the frozen spermatozoa is not the same as in fresh semen. % sperm fertility of A2 (2 arab hen) can be caused by inhibition of spermatozoa cells during their abilitity to reach the ovum, example: failure of spermatozoa to reach and enter the sperm storage tubule (SST), ability to reach the fertility site in the infundibulum, to penetrate the perivitelin layer of the ovum and failure of the spermatozoa to form the pronucleus that causes syngamy does not occur. Furthermore, (Shubash *et al*., 2005) explained that probable causes of fertility decline perhaps by the reduced capacity of SST to store spermatozoa and the induction of anti-sperm antibodies.

Mean of % sperm fertility of SK Kedu based this study was 72.22%. Sperm fertility on this result was not different from the % sperm fertility resulting from frozen semen sandhill crane from America using 10% concentration of DMSO with different extenders which is 73.9% (Blanco *et al*., 2012) and another result showed cryopreservation with 8% DMSO in Indian red jungle fowl was 73% sperm fertility (Rakha *et al*., 2018). The DMSO can maximize the replacement of water molecules in the cytoplasma, therefore formation of ice crystal is prevent and the cell membrane structure have a effective protection (Hu *et al*. 2015).

Furthemore, Yamashiro *et al*. 2010 explained that addition of exogenous substrate such as lactate improved sperm fertility in different spesies and may find lactate as suitable substrate for maintaining the energy production. Energy in sperm by ATP requires to maintain sperm fertility. ATP is hydrolyzed by the dynein adenosine triphosphatase, which converts the chemical energy of ATP into mechanical energy used for the movement of sperm (Storey, 2008). Egg yolk have the ability to maintain sperm fertility. Egg yolk have the beneficial molecules such as phospholipids and LDL which have a cryoprotective action to protect sperm against cold shock and the lipid-phase transition effect during the freeze–thaw process (Moussa *et al*., 2002).

But another, a result by used the lower concentration of DMSO was 4.5 % in White Leghorn showed 93% of sperm fertility in Beltsville Poultry Semen Extender (Voorst & Leenstra ,1995). Lower of % sperm fertility based on result could be due to the use of a higher DMSO concentration and the influence of the Lactated ringer's-egg yolk extender composition. Higher concentration of DMSO perhaps caused toxicity for sperm. Donoghue and Wishart, 2000) explained that several factors should be taken into consideration of cryoprotective agens toxicity to chicken sperm is cryoprotectant concentration, equilibration temperature, equilibration time, freezing rate, freezing method and post-thaw treatment. Further more, chicken sperm also have the difference morphology to mammalian sperm cells and affect in cryopreservation result. The filiform shape of the poultry sperm head is not much wider in diameter than the tail (Thurston & Hess, 1987). Therefore, sperm heads have less cytoplasmic volume, and this means they have less ability to move in the cryoprotectants (CPA). Less cytoplasmic volume and therefore have the less ability to move cryoprotectants inside the sperm head perhaps be one of the reasons avian spermatozoa do not survive the freezing process well (Donoghue & Wishart, 2000).

Sperm of poultry were not estimated to have the capacitation or motility hyperactivity during fertilization, although sperm of poultry have a long time in the oviduct before the mechanism oocyte penetration, which is more than 3 weeks in hens (Lemoine *et al*., 2008). During the fertilization time, the ovum will be ovulated consecutively at intervals of 24 hours or more in some species and can be fertilized 15 minutes after ovulation (Wishart & Staines, 1999).

Table 1: Composisition of lactated ringer's-egg yolk (LR-EY) extender

Constituent	mL
Lactated ringer's*	80.0
Egg yolk	20
pH	6.8
Total	100.0

*Lactated ringer's: Commercial solution (PT Emjebe Pharma)

Table 2: Extender composition of lactated ringer's-egg yolk with 10% of DMSO

Constituent	mL
LR-EY (%)	90
DMSO (%)	10
Penicillin (IU mL $^{-1}$)	1000
Streptomycin (mg mL $^{-1}$)	1
Total	100

Table 3. Sperm fertility using lactated ringer's-egg yolk with 10% of DMSO

Arab Hens	Total of Egg	Fertile Egg	Sperm fertility (%)
A1	9	7	77.78
A2	3	1	33.33
A3	6	5	83.33
Mean	**18**	**13**	**72.22**

Conclusions

% sperm fertility of A1 and A3 was higher than A2 which can be affected by AI time, extender, and concentration of cryoprotectan. The mean of % sperm fertility of SK kedu chicken after inseminated by frozen semen using lactated ringer's-egg yolk with 10% of DMSO was showed 72.22% and can maintain sperm fertility of SK kedu chicken.

References

Amira, L., Tainturier, D., Jeanneau, L., Thorin. C., Gerard ,O., Courtens, J.L., & Anton, M. 2004. Bull semen in vitro fertility after cryopreservation using egg yolk LDL: a comparison with optidyl, a commercial egg yolk extender. Theriogenology. 61(5):895-907.

Baskt, M. R., & Long, J. A. 2010. Techniques for semen evaluation, semen storage, and fertility determination. St Paul, Minnesota. The midwest poultry federation. 2:1-113.

Baskt, M. R., & Dymond, J. S. 2013. Artificial insemination in poultry. InTech. http://dx.doi.org/10.5772/54918.

Blanco, J. M., Long, J. A., Gee, G., Wildt, D. E., & Donoghue, A. M. 2012. Comparative cryopreservation of avian spermatozoa: Effects of freezing and thawing rates on turkey and sandhill crane sperm cryosurvival. Animal Reproduction Science. 131:1-8.

Blesbois, E., Grasseau, I., Seigneurin, F., Mignon-Grasteau, S., Saint, J. M., & Mialon-Richard, M.M. 2008. Predictors of success of semen cryopreservation in chickens. Theriogenology. 69: 252-61.

Blesbois, E. 2011. Frezing avian semen. Avian Biology Research 4: 52-58.

Brillard, J. P. 2003. Pratical aspects of fertility in poultry. World's Poultry Science Journal. (59): 441-446.

Cottler, F. M., Montgomery, M., & Theus J. 2009. Hematopoietic Stem Cell Transplantation in Clinical Practice. Churchill Living Stone. Pages: 249-256.

Diwyanto, K., Zainuddin, D., Sartika, T., Rahayu, S., Djufri., Arifin. C., & Cholil. 2011. Model pengembangan peternakan rakyat terpadu berorientasi agribisnis: komoditas ayam lokal. Bogor (ID): Direktorat Jenderal dengan Balitnak Ciawi.

Donoghue, A. M., & Wishart, G. J. 2000. Storage of poultry semen. Journal Animals Reproduction Science. 62: 213-232.

Froman, D. P. 2007. Sperm motility in birds: insights from fowl sperm. Soc Reproduction Fertility Supply 65:293-308.

Gadea, J. 2005. Sperm Factors Related to In Vitro And In Vivo Porcine Fertility. 63 (2005) 431-444.

Getachew, T. 2016. A review article of artificial insemination in poultry. World's Veterinary Journal. 6 (1): 26-35.

Holt, W. V. 2000. Fundamental aspects of sperm cryobiology: the importance of species and invidual differences. Theriogenology 53:47-58.

Hu, S., Zhu, Q. C., Han, C., Zhang, X. G., & Song, B. Y. 2015. Effects of different cryoprotectants on the cryopreservation of cattle testicular tissue. Archives Animal. Breeding. 58: 433-439.

Hubalek, Zdenek. 2003. Protectans used in the cryopreservation of microorganisms. Cryobiology 46: 205:229.

Jones, A. R. 1997. Metabolism of lactate by mature boar spermatozoa. Reproduction Fertility and Development. 9:227-232.

Kowalczyk, A. & Łukaszewicz, E. (2015) Simple and effective methods of freezing capercaillie (tetrao urogallus l.) semen. PLOS ONE 10: e0116797.

Krista, B., & Harianto, B. 2013. Jago Bisnis dan Beternak Ayam Kampung. Jakarta (ID): Agro Media Pustaka.

Lemoine, M., Grasseau, I., Brillard, J. P., & Blesbois, E. 2008. A reappraisal of the factors involved in in vitro initiation of the acrosome reaction in chicken spermatozoa. Reproduction Research. 136:391–9.

Lemoine, M., Mignon-Grasteau, S., Grasseau, I., Magistrini, M., & Blesbois, E. 2011. Ability of chicken spermatozoa to undergo acrosome reaction after liquid storage or cryopreservation. Theriogenology 75:122-130.

Lovelock, J. E. & Bishop, M. W. H. 1959. Prevention of freezing damage to living cells by dimethyl sulphoxide. Nature. 1394–1395.

McGann, L. E & Walterson, M. L. 1987. Cryoprotection by dimethyl sulphoxide and dimethyl sulfone. Cryobiology Vol 24 (1): 11-16.

Moussa, M., Martinet, V., Trimeche, A., Tainturier, D., & Anton, M. 2002. Low density lipoproteins extracted from hen egg yolk by an easy method: cryoprotective effect on frozen-thawed bull semen. Theriogenology, 57: 1695-1706.

Mulyono, R.H. & Pangestu, R.B. 1996. Analisis statistik UkuranUkuran Tubuh dan Analisis KarakterKarakter Genetik Eksternal pada Ayam Kampung, Ayam Pelung dan Ayam Kedu. Laporan Penelitian. Fakultas Peternakan. Institut Pertanian Bogor.

Nguyen, T. M. D., Alves, S., Grasseau, I., Métayer-Coustard, S., Praud, C., Froment, P., & Blesbois, E. 2014. Central Role of 5'-AMP-Activated Protein Kinase in Chicken Sperm Functions. Biology of Reproduction. 91(5)121:1-15.

Rakha, B. A., Ansari, M. S., Akhter, S., Zafar, Z., Naseer, A., Husaain, I., Blesbois, E., & Santiago-Moreno. 2018. Use of dimethylsulfoxide for semen cryopreservation in Indian red jungle fowl (*Gallus gallus murghi*). Theriogenology. Vol 122: 61-67.

Shubash, C. D., Naohiro, N., & Yukinori, Y. 2005. Effects of Repeated Artificial Insemination on the Structure and Function of Oviducal Sperm Storage Tubules in Hens. Journal of Poultry Science. 42: 39-47.

Sinurat, A. P., Santoso., Juarini, E., & Sumanto. 1992. Peningkatan produktivitas ayam buras melalui pendekatan sistem usahatani pada peternak kecil. Jurnal Ilmu Peternakan. 5(2):73-77.

Storey, B. T. 2008. Mammalian sperm metabolism: oxygen and sugar, friend and foe. The International Journal of Developmental Biology. 52:427–437.

Thurston, R.J. & Hess, B.L. 1987. Ultrastructure of spermatozoa from domesticated birds: comparative study of turkey, chicken, and guinea fowl. Scanning Microscope. 1, 1829–1838.

Van Voorst, A., & Leenstra, F. R., 1995. Effect of dialysis before storage or cryopreservation on fertilizing ability of fowl semen. Poultry Science. 74, 141–146.

Wishart, G. J., & Staines, H. J. 1999. Measuring sperm-egg interaction to access breeding efficiency in chickens and turkeys. Poultry Science.78:428-36.

Yamashiro, H., Toyomizu, M., Kikusato, M., Toyama, N., Sugimura, S., Hoshino, Y., Abe, H., Stefan, M., & Sato, E. 2010. Lactate and Adenosine Triphospate in the Extender Enhance the Cryosurvival of Rat Epididymal Sperm. Journal of the American Association for Laboratory Animal Science. 49(2): 160–166.

Zhang, S. S., Hu, J. H., LI, Q. W., Jiang, Z. L. & Zhang, X. Y. 2009. The cryoprotective effects of soybean lecithin on boar spermatozoa quality. African Journal of Biotechnology. 8: 6476-6480.