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Research Article
Effect of Different Levels of Dimethylacetamide (DMA) on Sperm Quality of Bangkok Rooster Chicken and Sperm Survivability in Reproductive Tract of Hen

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Abstract

Objective: The objective of this study was to determine the effect of different dimethylacetamide (DMA) levels on sperm quality of Bangkok rooster chicken and sperm survivability in reproductive tract of hen. **Materials and Methods:** Sperm was collected from 4 Bangkok rooster chicken aged 1 year. Thirty native hens were used for insemination. Sperm from roosters was collected once a week and cryopreserved 24 h in liquid nitrogen containers at -196°C using 3% (P1), 5% (P2), 10% (P3), 14% (P4) and 18% (P5) DMA levels. Frozen thawed sperm quality was observed after thawing at 4°C for 60 sec. The hens were slaughtered and sperm viability was recorded in their reproductive tract on 3, 7, 14 and 21 days after insemination. **Results:** The results showed that freezing decreased the sperm motility, viability and increased abnormalities. The P2 was observed to be the best DMA concentration for chicken sperm cryopreservation. Different DMA concentrations significantly ($p<0.01$) affected the motility, viability and abnormalities of frozen thawed sperm. Sperm motility (%) after thawing in P1, P2, P3, P4 and P5 were 38.00±9.08, 46.00±8.94, 16.00±5.48, 8.00±2.24 and 1.00±2.74, respectively. Sperm viability (%) after thawing was 40.10±7.21, 53.50±12.53, 22.00±4.43, 15.30±11.40 and 12.00±3.98 in P1, P2, P3, P4 and P5, respectively. Sperm abnormalities (%) after thawing in P1, P2, P3, P4 and P5 were 60.40±7.40, 41.00±3.32, 67.20±4.09, 68.20±8.58 and 71.00±11.64, respectively. In P2, spermatozoa from the hen's reproductive tract (vagina, uterus, infundibulum and fimbria) were found motile up to 21 days after insemination. **Conclusion:** It can be concluded that the best DMA concentration for chicken sperm cryopreservation was 5% level in terms of sperm quality and its survivability in the reproductive tract of hen.

Key words: Frozen sperm, Bangkok chicken, sperm quality, survivability, reproductive tract

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Chicken sperm has a unique characteristic, it has high concentration spermatozoa in a limit ejaculation volume. This characteristic could be processed into commercial product, for example freezing sperm. Freezing sperm could damage the spermatozoa because of cold shock. So, a cryoprotectant agent (CPA) is used to protect sperms from freezing damage. Dimethylacetamide (DMA) is the one of common CPA for chicken sperm cryopreservation that gives the best results[1]. The DMA is usually used in the preparation of creating a synthetic membrane like cellulose membrane[2]. Acetate compound in DMA is effective to separate ions in the reverse osmoses process[3].

Dimethylacetamide is a z common CPA that is being used in chicken sperm cryopreservation at various levels. Six percent DMA level has been reported to be useful in chicken sperm cryopreservation[4-6]. Different membrane characteristics of spermatozoa demanded the difference of DMA levels for cryopreservation. Chicken spermatozoa have a great permeability membrane compared with spermatozoa of other species as it has low rigidity that affects the mobility of cellular substances to diffuse across the spermatozoa membrane[4].

Artificial Insemination (AI) could increase the amount of fertile eggs and reduce the usage of roosters, therefore, the freezing sperm could be one of the alternatives to support the AI. One insemination with fresh sperm could produce fertile eggs up to 21 days[7]. However, fertilizing ability in chicken that are inseminated with frozen sperm and how the spermatozoa from frozen semen survive in female reproductive tract also are still unknown. Many factors can affect fertility, especially the levels of CPA. Therefore, the present study was undertaken to evaluate the effect of different DMA levels in chicken sperm cryopreservation and sperm survivability in female reproductive tract of hen.

MATERIALS AND METHODS

Animal: Four rooster Bangkok chicken aged 1 year were procured from traditional farm in Gunung Kidul region, Yogyakarta as sperm donor and for insemination 30 native laying hen chicken from traditional farm in Sleman region, Yogyakarta were used in this study. The birds were housed in individual battery cages. The roosters received 100 g day^{-1} commercial feed and the hens received *ad libitum* access to a diet containing layer concentrate feed, corn (mash) and rice bran with ratio 3:4:3.

Semen cryopreservation and insemination: Pooled semen from roosters was collected once a week by the dorso-abdominal massage. Single ejaculates were visually examined and transferred into a glass tube. Each freshly collected semen sample was evaluated for motility, viability and abnormalities. Semen was diluted with modification of Betsville Poultry Semen Extender (BPSE) at concentration 0.5×10^9 million mL^{-1}, mixed with 3% (P1), 5% (P2), 10% (P3), 14% (P4) and 18% (P5) dimethylacetamide (DMA, Germany) in the final concentration. The mixture was then equilibrated at 5°C for 60 min, transferred into 0.25 mL plastic polycarbonate straws and vitrified 10 cm above N_2 fumes for 5 min and placed immediately in goblet and then plunged into liquid nitrogen at -196°C (taylor and wharton container having capacity of 20 L). Semen samples were thawed in 4°C water bath for 60 sec before insemination and evaluated for motility, viability and abnormalities. Insemination was done used 1 mL syringe inserted into the vagina. Insemination was performed in a maximum of 30 min after oviposition.

Sperm viability in female reproductive tract: The hens were slaughtered at 3, 7, 14 and 21 days after insemination and female reproductive tracts were collected. Three hens were used in each slaughter. Flushing in female reproductive tract (vagina, uterus, infundibulum and fimbria) was done with physiological NaCl solution to determine the sperm survivability under electronic microscopic (Nikon 120, Japan) at 40x magnification.

Statistical analysis: The data obtained were described as the Mean ± SD and analyzed with one way ANOVA. Signifcant data was evaluated by Duncan's Multiple Range Test (DMRT) with differences considered significant when the probability was less than 5%.

RESULTS

The process of semen cryopreservation caused unfavorable changes in the motility, viability and abnormality of spermatozoa (Fig. 1). The percentage of motile cells decreased significantly ($p<0.01$) by freezing process in P1, P2, P3, P4 and P5 from 85-38, 77-46, 70-16, 63-8 and 23-1%, respectively. Best sperm motility was observed in P2 with worst motility in P5 after thawing. Further, the sperm viability after freezing-thawing decreased significantly ($p\leq0.05$) in P1, P2, P3, P4 and P5 from 75-40, 76-53, 61-22, 30-15 and 18-12%, respectively. Likewise, the sperm abnormalities after thawing increased significantly ($p\leq0.01$)

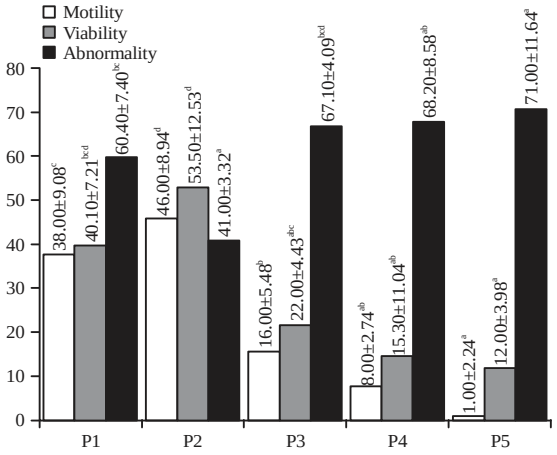

Fig. 1: Quality of chicken spermatozoa after thawing cryopreserved with various levels of DMA. [a-d]Means with different superscripts, the data were differ significantly at $p<0.01$

Table 1: Survivability of DMA cryopreserved sperm in the female reproductive tract of inseminated hen up to 21 days

Slaughtered days	V	U	I	F
3	-	-	√	-
7	√	-	√	-
14	√	√	√	√
21	√	-	√	-

V: Vagina, U: Uterus, I: Infundibulum, F: Fimbria, -: Sperm not found, √: Sperm found

after thawing in P1, P2, P3, P4 and P5 from 18-60, 18-41, 25-67, 64-68 and 68-71%, respectively. The sperms in the reproductive tract of hens were easily found on 3 and 7 days after insemination but were difficult to locate on 14 and 21 days after insemination and warranted higher magnification under microscope. Three days after insemination, the sperms were found only in infundibulum, 7 days after insemination found in vagina and infundibulum, 14 days after insemination found in vagina, uterus, infundibulum and fimbria and 21 days after insemination found in vagina and infundibulum (Table 1).

DISCUSSION

In the present study, the freezing caused cold shock in spermatozoa. There occurs a drastic temperature change during freezing process, therefore, cryoprotectants are used to protect sperms. However, adding the cryoprotectants caused higher osmotic pressure on the sperm diluter affects the balance between intracellular and extracellular fluid. The P1 generated low concentrated diluter, while P3, P4 and P5 resulted in more concentrated diluter. However, P2 gave the best results than other treatments. The addition of 3% DMA (P1) gave a low osmotic pressure but failed in protecting sperm from freezing cold shock, thereby justifying the lower motility and viability when compared with P2. The difference in the density affected the osmotic pressure of the diluters. Sperms can only survive in certain osmotic pressure and 350 mOsmol kg^{-1} is the best osmolarity to keep the sperm viability[8]. Thus, any change in osmotic pressure would interfere with sperm metabolism and decreases the quality of sperms. The DMA addition 6-9% resulted in the osmotic pressure of 1143-1576 mOsmol kg^{-1} and 12% gave 2100 mOsmol kg^{-1} [9]. Fluid imbalance in sperm cells caused the cells to become dehydrated during freezing, resulting in the death of spermatozoa. Dehydration results in hyperosmotic environment because cellular substances diffuse out of the cells and disturb the metabolism process[4]. Decreasing of sperm quality in high osmotic pressure occurs by different hydrostatics and decreased effective area for bonding (Hydrogen+protein+end polar of phospolipid sperm membrane) when sperm cell dehydrates[9].

Insemination was performed maximum in 30 min after oviposition because there were contractions of female reproductive tract that relate with oviposition[10]. Mesotocin (MT) reported have been associated to have an effect to enhance the inducing oviposition by arginine vasotocin (AVT) and the effect of the enhancement by MT on AVT-induced oviposition may be carried by an increase in the binding affinity of the AVT receptor in the uterus[11]. Spermatozoa were found in the female reproductive tract up to 21 days after insemination using frozen sperm. Spermatozoa survived up to 21 days and were stored in Sperm Storage Tubules (SSTs) of the hen, which is a part of Utero Vaginal Junction (UVJ). About 7-10 days after insemination or mating, the sperm reaches the vagina and is stored in SSTs[12]. This correlates with the results of the present study that why the sperms were only found in vagina 3 days after insemination. Spermatozoa were found in infundibulum on 7, 14 and 21 days after insemination. Sperm are released from SSTs and moved into infundibulum, where the fertilization takes place[13]. Spermatozoa that have already undergone capacitation, wait for ovum to fertilization in the infundibulum. This relates with the results of present study that why spermatozoa were always found in infundibulum. Spermatozoa were found up to 21 days insemination, thus there was no difference between frozen and non-frozen sperm regarding ability to survive in the female reproductive tract. One important factor supporting sperm storage in the SSTs is defense from anti-sperm immune

responses in the oviduct. The elimination of anti-sperm immune responses by the transforming growth factor β (TGFb) system has been reported as one of the factors responsible for sperm maintenance in the SSTs[14].

CONCLUSION

It can thus be concluded that the 5% DMA cryoprotectant concentration resulted in better sperm quality after thawing. Further, the spermatozoa in 5% DMA group were found in the female reproductive tract (vagina, uterus, infundibulum and fimbria) up to 21 days after insemination.

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