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Study of the quality and integrity of spermatozoa acrosome caps in frozen sexing semen Friesian Holstein cattle

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ABSTRACT: Artificial insemination (AI) of dairy cows using sexing semen is the right choice to obtain female calves as *replacement stock*. The success of AI is influenced by the quality of the spermatozoa used. The purpose of this study was to determine the quality of semen produced by Lembang Artificial Insemination Center. The material used is 30 frozen semen straws of FH cattle produced by Lembang Artificial Insemination Center. This research method uses observational methods. The research variables included individual motility, viability, abnormalities, concentration, total motile spermatozoa, and intact acrosome caps of spermatozoa. The data obtained were analyzed by the Analysis of Variance (ANOVA) test and continued with Duncan's test if significantly different. The results showed that individual motility, concentration, total motile spermatozoa, and intact acrosome caps of spermatozoa had a very significant effect on all treatments ($P<0.01$), while spermatozoa abnormalities had a significant effect ($P<0.05$) on all treatments, but on viability, there was no effect ($P> 0.05$) in all treatments. Motility of individual spermatozoa in non-*sexing* semen (55.80±4.56)%, sperm X (46.30±5.36)%, sperm Y (46.70±6.40)%. Viability of spermatozoa in non-*sexing* semen (74.57±4.05)%, sperm X (73.24±2.30)%, sperm Y (72.51±2.68)%. Spermatozoa abnormalities in non-*sexing* semen (2.73±2.24)%, sperm X (4.94±1.57)%, sperm Y (3.25±2.72)%. Concentration of non-*sexing* semen (33.88±1.98 million/mini straw), sperm X (25.06±4.29 million/mini straw), sperm Y (25.13±5.74 million/mini straw). The number of sperm motile non-sexing (18.60±1.58 million/mini straw), sperm X (11.68±2.82 million/mini straw), sperm Y (11.64±2.68 million/mini straw). Intact acrosome caps, non-sexing semen (78.40±4.84)%, sperm X (74.08±3.80)%, sperm Y (70.70±4.78)%. The conclusion in this study was that the quality of post thawing of non sexing frozen semen was higher of sexing frozen semen using BSA method.

Keywords: Sexing; Artificial insemination; Semen quality; Acrosom integrity

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INTRODUCTION

In the dairy farming industry, farmers expect the birth of female calves as substitute livestock that will replace the mother to produce milk (Wahjuningsih *et al*., 2019). Determining the sex of a calf before birth has an economic advantage, namely being able to reduce maintenance costs and support breeding programs in the selection of superior breeds (Putri *et al*., 2015). Reproductive technology that can be used to produce calves of certain sex and has the potential to control livestock mating to allow more regular calf births is *sexing* (Saili *et al*., 2017). Artificial Insemination (AI) using *sexing* semen can provide benefits to the dairy farming industry (Boro *et al*., 2016). One of the dairy cattle breeds that are commonly kept by the Indonesian people is the Friesian Holstein (FH) dairy cow. FH cattle can adapt well to the tropics so this type of cattle is suitable for breeding in Indonesia. The advantage of FH cows is that their milk production is the highest compared to other dairy cattle breeds (Gertenbach, 2005). The milk production of FH dairy cows in their country can reach 6.000-8.000 kg/head/lactation (Ratnasari *et al*., 2019).

Research results in Mahfud *et al*. (2019) stated that non-*sexing* semen and Y spermatozoa resulting from non-*sexing* after thawing had motility of 36.00% and 31.40%, the viability of 81.70% and 75.89%, abnormalities of 6.93% and 6.78%, the concentration of spermatozoa was 31.67 million/mini staw and 12.125 million/mini straw and total motile spermatozoa were 11.39 million/mini straw and 5.10 million/mini straw. Semen quality is one of the factors that can affect the success of AI (Susilawati, 2011). Therefore, this study aims to determine the quality of frozen non-sexing and sexing semen produced by Lembang Artificial Insemination Center.

MATERIALS AND METHODS

Time and Place of Research

This research was conducted on August 6, 2021. Spermatozoa quality test was conducted at the Reproduction Laboratory of the Faculty of Animal Science, Brawijaya University, Malang.

Material and Methods

The materials used in this study were 30 straws of frozen semen of FH bull produced by Lembang Artificial Insemination Center consisting of 10 straws of non-*sexing*, 10 straws of sperm X, and 10 straws of sperm Y. Frozen sexing semen produced by Lembang Artificial Insemination Center used the column *Bovine Serum Albumin* (BSA) method. The observed variables consisted of motility, viability, abnormality, concentration, total motile spermatozoa (TSM), and intact acrosome caps of spermatozoa.

Motility of Spermatozoa

Motility is obtained by dropping a drop of semen on the object glass and covered with cover glass. The motility was observed using a microscope with 400x magnification. Motility can be observed in at least 5 fields of view (Susilawati, 2011).

Viability of Spermatozoa

Viability is obtained by dropping one drop of semen on the object glass, then mixed with one drop of eosin-negrosin dye. After that, it will be smeared using a glass object with 45 degrees deviation. The viability was observed using a microscope with 400x magnification. (Susilawati, 2011). And then, it is calculated using the formula:

$$\text{Viability (\%)} = \frac{\text{total motile spermatozoa}}{\text{total spermatozoa}} \text{ x } 100\%$$

(Dethan *et al*., 2010)

Abnormality of Spermatozoa

The preparation used to calculate the viability of spermatozoa was continued to calculate abnormality. The abnormality was observed using a microscope with 400x magnification. And then, it is calculated using the formula:

$$\text{Abnormalitas (\%)} = \frac{\text{total abnormal spermatozoa}}{\text{total spermatozoa}} \text{ x } 100\%$$

(Barek *et al*., 2020)

Concentration of Spermatozoa

The concentration of spermatozoa can be determined by taking 10 µl of semen using a micropipette, then putting it into an Eppendorf tube filled with 990 µl NaCl 3% and homogenized for 2-3 minutes. After that, it was dripped on a *hemocytometer* and observed for five large squares, 4 in the corner and 1 in the middle. And then, it is calculated using the formula:

$$\text{Concentration} = \text{N X 5 X dilueten factor X } 10.000$$

(Nalley *et al*., 2016).

Total Motile Spermatozoa

Total motile spermatozoa were calculated by multiplying the percentage of individual motility by the total concentration of spermatozoa in million/ml. The formula is:

TSM=Motile Spermatozoa X Spermatozoa concentracion

(Susilawati, 2011).

Acrosome Integrity (AIn)

10 µl semen was taken and mixed with 990 µl formol-saline solution until homogenized. Incubated for 5 minutes. Then make preparations on the object glass and covered it with cover glass. After that, observed using a microscope with 400x magnification (Susilowati, 2010). The calculation of the percentage of acrosome integrity was using the formula:

$$\text{AIn (\%)} = \frac{\text{Intact acrosom of spermatozoa}}{\text{Total spermatozoa}} \text{ x } 100\%$$

(Sitepu *et al*., 2021).

Statistical Analysis

Data obtained were analyzed by Analysis of Variance (ANOVA) and continued with Duncan's test if significantly different. Furthermore, the variable motility and total motile spermatozoa were analyzed by Chi-Square with the expected value according to SNI.

RESULT AND DISCUSSION

Evaluation of semen after thawing was important to assess the success of the freezing process and to maintain the frozen semen standards that were produced by the center before being distributed to dairy cow breeders. The quality post thawing of FH cattle spermatozoa is presented in Table 1.

Table 1. The quality of FH non-sexing and sexing frozen semen

Parameters	Treatments		
	Non-*sexing* (Average ± SD)	*Sexing* X (Average ± SD)	*Sexing* Y (Average ± SD)
Individual Motility (%)	55,80±4,56[b]	46,30±5,36[a]	46,70±6,40[a]
Viability (%)	74,57±4,05	73,24±2,30	72,51±2,68
Abnormalities (%)	2,73±2,24[a]	4,94±1,57[b]	3,25±2,72[ab]
Concentration (Million)/mL	33,88±1,98[b]	25,06±4,29[a]	25,13±5,74[a]
Total Motile Spermatozoa (Million)/mini straw	18,60±1,58[b]	11,68±2,82[a]	11,64±2,68[a]
Intact Acrosome Cap (%)	78,40±4,84[b]	74,08±3,80[ab]	70,70±4,78[a]

Different lowercase letters on the same line are significant (P<0.05).

Individual Motility of Spermatozoa in Non-Sexing and Sexing Frozen Semen

Motility is the movement of spermatozoa. Spermatozoa are said to be motile if they move forward progressively. Individual motility is one of the important indicators in determining the fertility of a male.

Table 1. shows that the percentage of individual motility of FH cattle spermatozoa obtained in this study averaged between 46-55%, this figure was higher than the expected value of 40%. The results of statistical analysis showed that between treatments showed a very significant effect (P<0.01) on the motility of individual spermatozoa. The percentage of individual motility of non-*sexing* was significantly higher (P<0.05) compared to sperm X and sperm Y, while there was no significant difference between sperm X and sperm Y (P>0.05).

The motility of *sexing* semen showed a lower value than non-*sexing* semen. The decrease in individual motility was thought to be due to the influence of the centrifugation process during the separation of spermatozoa. According to Mustofa *et al*. (2020) that centrifugation performed to separate spermatozoa causes friction between spermatozoa and the diluent medium, spermatozoa with each other, and test tubes.

Centrifugation also causes damage to the spermatozoa membrane, so when the spermatozoa membrane is damaged it can trigger an increase in free radicals (Bondan *et al*., 2016). The presence of these free radicals affects the physiological regulation of spermatozoa during capacitation and hyperactivation (Aitken *et al*., 2012). According to Susilawati (2004), fresh semen after the separation of spermatozoa and the freezing process will experience a decrease in motility. The decrease in motility will affect the ability to fertilize and have an impact on the development of the embryo (Khalil *et al*., 2018).

Viability of Spermatozoa in Non-Sexing and Sexing Frozen Semen

Viability is the ability of spermatozoa to survive. Spermatozoa viability was observed using *eosin negrosin* staining. The live spermatozoa are colorless, indicating that the spermatozoa don't absorb the dye, while the dead spermatozoa are red-purple because the spermatozoa absorb the dye.

Table 1. shows that the percentage of spermatozoa viability of FH cattle obtained in this study averaged between 72-74%, this figure was classified as suitable for AI because it was above 50%. Descriptively, the viability of spermatozoa between treatments was not much different and statistically showed that between treatments had no significant effect (P>0.05) on spermatozoa viability. Thawing performed on non-*sexing*, sperm X, and sperm Y were not significantly different (P>0.05).

The highest spermatozoa viability was found in non-*sexing* at 74.57±4.05% and the lowest was in sperm Y at 72.51±2.68%. Spermatozoa viability after separation decreased. This is due to the mechanical influence of centrifugation on the separation

process which causes friction between spermatozoa with the medium and fellow spermatozoa, resulting in cell membrane damage (Mahfud *et al*., 2019). The viability of spermatozoa is highly dependent on the integrity of the spermatozoa membrane. Spermatozoa that experience membrane damage will result in disruption of the intracellular metabolic processes of spermatozoa so that the ability of spermatozoa to survive will be weakened and even cause the death of spermatozoa (Zelpina *et al*., 2012).

Abnormalities of Spermatozoa in Non-Sexing and Sexing Frozen Semen

Abnormality is a condition in which spermatozoa experience physical abnormalities from normal conditions. It is important to observe spermatozoa abnormalities in assessing the quality of spermatozoa because if the abnormality is high, then the fertility rate is low so it has an impact on the fertilization process during copulation.

Table 1. shows that between treatments had a significant effect (P<0.05) on spermatozoa abnormalities. The percentage of spermatozoa abnormalities in non-*sexing* was significantly different (P>0.05) with sperm X, but sperm Y was not significantly different (P<0.05) with non-*sexing* and sperm X. The lowest spermatozoa abnormality in this study is frozen semen non-*sexing* at 2.73±2.24% and the highest was in the sperm X at 4.94±1.57%.

The presence of abnormalities in semen after *sexing* is thought to be due to the friction between spermatozoa and the tube wall during the separation process, causing damage to the morphology of spermatozoa (Anwar *et al*., 2019). In addition, spermatozoa abnormalities can also be caused by damage to the spermatozoa membranes during the cooling, freezing, and storage of frozen semen. This is by Sholihati *et al*. (2008) who state that spermatozoa abnormalities have increased during the cooling and freezing process caused by cold stress so that the osmotic pressure becomes unbalanced due to metabolic processes that continue during storage. Spermatozoa that are damaged in the membranes of the head cause a decrease in fertility, while damage to the tail causes a decrease in the ability of the spermatozoa to move (Prihantoko *et al*., 2020).

The percentage of spermatozoa abnormalities in this study ranged from 2-4% and was categorized in the normal range. Semen that has good quality has an abnormality percentage below 20% (Purwantara *et al*., 2010).

The concentration of Spermatozoa in Non-Sexing and Sexing Frozen Semen

Spermatozoa concentration is the number of spermatozoa in one ml of semen. Based on Table 1. it is known that the average concentration of frozen semen of non-sexing, sperm X, and sperm Y is 33.88±1.98 million/mini straw, 25.06±4.29 million/mini straw, and 25.13±5.74 million/mini straw. The concentration of frozen semen non-*sexing* was higher than *sexing* sperm and statistically showed that between treatments had a very significant effect (P<0.01) on the concentration of spermatozoa. The concentration of frozen semen of non-s*exing* was significantly higher (P<0.05) than sperm X and sperm Y, while the concentration between sperm X and sperm Y was not significantly different (P>0.05). The difference in concentration decrease between non-*sexing* and *sexing* sperm is thought to be due to the *filling* and *sealing* during packaging because at the time of testing there were straws with air cavities.

Total Motile Spermatozoa in Non-Sexing and Sexing Frozen Semen

Total motile spermatozoa are the number of spermatozoa that are considered fertile based on the result of multiplying the total concentration of spermatozoa with progressive motility of spermatozoa. The optimal total motile spermatozoa will help the chances of fertilization be higher.

Based on Table 1. shows that the total motile spermatozoa obtained in this study averaged between 11-18 million/mini straw. The total value of motile spermatozoa in all treatments met the SNI standard as AI semen because it had total motile

spermatozoa above 10 million/mini straw. Statistically, it was seen that between treatments had a very significant effect (P<0.01) on the total motile spermatozoa. Total motile spermatozoa in non-*sexing* frozen semen were significantly higher (P<0.05) than sperm X and sperm Y, while the total motile spermatozoa between sperm X and sperm Y was not significantly different (P>0.05).

The decrease in the total number of motile spermatozoa in *sexing* sperm is caused by the centrifugation process during the separation process. In this process, spermatozoa require more energy so that their physiological conditions remain normal. If this energy cannot be met, then the motility of spermatozoa will decrease and result in death (Aji *et al*., 2017). Decreased motility can be a factor in the low total motile spermatozoa. This is by (2013) who explained that the total motile spermatozoa were influenced by the motility of the individual and the number of spermatozoa.

Acrosome Integrity of Spermatozoa in Non-*sexing* and *Sexing* Frozen Semen

The intact acrosome hood is a layer found on the head of the spermatozoa which serves to protect the release of genetic material from the head of the spermatozoa. The assessment of the quality of spermatozoa is not only seen from the motility but also from the integrity of the acrosomal hood of the spermatozoa.

The results of the quality test in Table 1. show that between treatments had a very significant effect (P<0.01) on the intact acrosome cap. The percentage of intact acrosome cap on semen non-*sexing* was significantly different (P>0.05) with sperm Y, but sperm X was not significantly different (P<0.05) with non-*sexing* and sperm Y. The lowest intact acrosomal cap found in sperm Y was 70.70±4.78% and the highest was in the non-*sexing* treatment at 78.40±4.84.

The acrosome caps of sexing semen spermatozoa overall showed lower numbers than non-*sexing.* According to Dasrul (2005) that the decrease in the quality of spermatozoa is influenced by chemicals in the medium and the mechanical effect of friction between the membrane surface and the particles of the medium or the tube wall. Susilowati (2010) added that centrifugation causes damage to the spermatozoa membrane so that the flow of ion traffic is disrupted and causes changes in lipid composition, where lipids play an important role in maintaining the integrity of the spermatozoa membranes

CONCLUSIONS

The post thawing quality of non sexing frozen semen was heigher than that of sexing frozen semen (sperm X and sperm Y) using the BSA method, motility of individual spermatozoa in non *sexing* semen 55.8%, sperm X 46.3%, sperm Y 46.70%. Viability of spermatozoa in non *sexing* semen 74.57%, sperm X 73.24%, sperm Y 72.51%. Abnormality of spermatozoa in non *sexing* semen 2.73%, sperm X 4.94%, sperm Y 3.25%. Concentration of non *sexing* semen 33.88 million/mini *straw*, sperm X 25.06 million/mini *straw*, sperm Y 25.13 million/mini *straw*. The number of sperm motile non *sexing* 18.60 million/mini *straw*, sperm X 11.68 million/mini *straw*, sperm Y 11.64 million/mini *straw*. Intact acrosome caps, non *sexing* semen 78.40%, sperm X 74.08%, sperm Y 70.70%.

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REFERENCES

Aitken, R. J., Jones, K. T., & Robertson, S. A. (2012). Reactive oxygen species and review sperm function-in sickness and in health. *J. Androl.* 22: 1096-1106.

Aji, R. N., Panjono., Agus, A., Widyobroto, B. A., Hartatik, T., Budisatria, I. G. S., Ismaya., & Bintara, S. (2017). Kinerja reproduksi sapi betina sumba ongole yang diinseminasi dengan semen beku

sapi jantan belgian blue. *Buletin Peternakan*. 41(4): 379–384.

Anwar., Solihati, N., & Rasad, S. D. (2019). Pengaruh medium dan lama inkubasi dalam proses *Non-sexing* sperma terhadap kualitas semen kambing boer. *Jurnal Ilmu Ternak*. 19(1): 53-61.

Barek, M. E., Hine, T. M., Nalley, W. M., & Belli, H. L. (2020). Pengaruh penambahan sari wortel dalam pengencer sitrat kuning telur terhadap kualitas spermatozoa kambing bligon. *Jurnal Nukleus Peternakan*. 7(2): 109-117.

Boro, P., Naha, B. C., Madkar, A., & Prakash, C. (2016). Sexing of semen in bulls: a mini-review. *Inter. J. Appl. Res.* 2(4): 460-462.

Bondan, C., Zanella, E., Zanella, R., Poetini, M. R., Marques, M. G., & Soares, J. C. M. (2016). Oxidative status of boar semen during storage. *American Journal of Biochemistry and Biotechnology*. 12(2): 95-101.

Dasrul. (2005). Peran senyawa oksigen reaktif dalam mekanisme kerusakan integritas membran spermatozoa kerbau lumpur hasil sentrifugasi gradien densitas percoll. Disertasi. Program Pascasarjana. Universitas Airlangga.

Dethan, A. A., Kustono., & Hartadi, H. (2010). Kualitas dan kuantitas sperma kambing bligon jantan yang diberi pakan rumput gajah dengan suplementasi tepung darah. *Buletin Peternakan*. 34(3): 145- 153

Gertenbach, W. D. (2005). Breed of dairy Cattle. *J. Anim Sci*. 13(3): 277-286.

Khalil, W. A., El-Harairy, M. A., Zeidan, A. E. B., Hassan, M. A. E., & Elsaeed, O. M. (2018). Evaluation of bull spermatozoa during and after cryopreservation: structural and ultrastructural insights. *International Journal of Veterinary Science and Medicine*. 6: S49–S56.

Nalley, W. M., Pratama, A., & Arifiantini, I. (2016). Pengujian motilitas dan validitas konsentrasi spermatozoa dalam kemasan semen beku dari salah satu balai inseminasi buatan daerah (BIBD) Indonesia. Seminar Nasional Laboratorium Riset Terpadu ke-1 Undana, 12-13 Agustus 2016. Kupang: Universitas Nusa Cendana.

Prihantoko, K. D., Kusumawati, A., Widayati, D. T., & Pangestu, M. (2020). Effects of storage duration on mitochondrial activity and DNA fragmentation of post-thawed spermatozoa from several ongole grade bull in Indonesia. *Veterinary Practitioner*. 21(2):264-268.

Purwantara, B., Arifiantini, R. I., & Riyadhi, M. (2010). Sperm morphological assessments of friesian holstein bull semen collected from three artificial insemination centers in Indonesia. *Journal Indonesian Trop. Anim. Agric*. 35(2). 90-94.

Putri, R. D. A., Gunawan, M., & E. M. Kahn. (2015). Uji kualitas sperma *non-sexing* sapi friesian holstein (FH) pasca thawing. *Pros Sem Nas Masy Biodiv Indon.* 1(8): 2057-2061.

Ratnasari, D., Atabany, A., Purwanto, B. P., & Salma, L. B. (2019). Growth patterns of holstein friesian dairy cow (FH) from brith to first child based on mathematical analysis of the gomperts model. *Bulletin of Animal Science*. 43(3): 184-187.

Mustofa, R., Yekti, A. P. A., Aryogi., Pamungkas, D., Prafitri, R., Huda, A. N., Kuswati., & Susilawati, T. (2020). Karakter motilitas spermatozoa hasil *Non-sexing* pada sapi peranakan ongole dengan volume awal yang berbeda. *Jurnal Agripet*. 20(2): 210-215.

Saili, T., L. O. Nafiu, L. O. B., & Rahadi. (2017). Efektivitas sinkronisasi estrus dan fertilisasi spermatozoa hasil *Non-sexing* pada sapi bali di Sulawesi Tenggara. *Jurnal Veteriner*. 18(3): 353-359.

Sitepu, S. A., & Marisa, J. (2021). Persentase tudung akrosom utuh spermatozoa pada semen beku sapi simmental dengan penambahan gentamisin dan minyak atsiri jeruk

manis pada bahan pengencer. Prosiding Seminar Nasional Fakultas UNS. 5(1).

Susilawati, T. (2004). Keberhasilan IB menggunakan sexing semen setelah dibekukan. Seminar Nasional Teknologi Peternakan dan Veteriner.199–202.

Susilawati, T. (2011). *Spermatology*. Malang. Universitas Brawijaya Press.

Wahjuningsih, S., Susilawati, T., Suyadi., Ihsan, M. N., Busono, W., Isnaini, N., & Yekti. (2019). *Teknologi Reproduksi Ternak*. Malang: UB Press. ISBN: 978-602-432-843-6.