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The Quality of Post Thawing Spermatozoa of Indigenous Indonesia Cattle Using Ice Cube and Salt as an Alternative of Liquid Nitrogen

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Abstract**—** *The main obstacle in the implementation of Artificial Insemination (AI) is the absence of cheap and effective field tools or flasks that can maintain the quality of frozen sperm during AI implementation. So far, inseminators always use flasks with liquid nitrogen to bring frozen straw into the field. So that it is higher cost and ineffective. The purpose of this study was to determine the storage time of frozen sperm in a field thermos with ice cooling and table salt in various ratios of the effect on the quality of semen from Pasundan cattle from West Java. The research be carried out from March to August 2021 at the Balai Inseminasi Buatan (BIB) Lembang, West Java and the Laboratory of Biotechnology and Reproduction, Universitas Mercu Buana Yogyakarta. The research used frozen semen straws in the form of mini straw from the Pasundan cattle breed, which was produced by the BIB Lembang. The research used an experimental method with a completely randomized design with a unidirectional pattern, namely ice and salt ratio with 3 replications. T0 for control, T1 for ice cubes + 30% salt, T2 for ice cubes + 40% salt and T3 for ice cubes + 50% salt. Each treatment is stored for 0, 2, 4, 6 hours. To determine the difference in motility in the treatment of the ratio of ice and salt in a field flask, analysis of variance was carried out. If there is a difference, continue with the Duncan's Multiple Range Test (DMRT) difference test. The use of storage media filled with liquid nitrogen had a higher motility rate ($p<0.05$) compared to a mixture of ice cubes and salt (T1, T2 and T3). The use of liquid nitrogen (T0) was higher viability ($p<0.05$) compared to a mixture of ice cubes and salt (T1, T2 and T3). However, the use of ice cubes and salt at all levels (T1, T2 and T3) showed no significant difference ($p<0.05$). Viability of spermatozoa decreased by 29.62%. The results showed that the average mass movement of spermatozoa ranged between good and sufficient categories, this could occur because treatment using salt and ice caused cold shock. Extreme temperature changes that occur, namely from a temperature of -196^{0}C (liquid nitrogen temperature) to the temperature of the ice cube and salt treatment in the thermos, which is -15^{0}C, make most of the spermatoza dead. T3 shown the best results for the variable motility, viability and wave motion.*

Keywords**—** *Pasundan Cattle, Ice cube, Salt, Quality of spermatozoa*.

I. INTRODUCTION

The development of the livestock sub-sector plays a crucial role in supplying the nutritional needs of the Indonesian people, which in 2004 amounted to more than 220 million. The provision of nutritional needs derived from livestock products such as eggs, meat, and milk has not been fully provided domestically. Some products such as meat and milk still have to import from abroad (Anonymous, 2000). The development of the integrated livestock sub-sector at this time must be directed to the orientation of agrAIusiness and agro-industry. In line with this program, the use of superior seeds is necessary to ensure business continuity, both in productivity and business sustainability. The fulfillment of community needs for quality and sustainable livestock meat is really a solution to a problem that exists both at the community level and at the national level (Anonymous, 2000).

The appearance of livestock potential is fundamentally influenced by two main factors: genetic and environmental factors, including the overall maintenance management. It is known that the environment and adequate management handling or following the needs of the livestock will not give the expected production expression (quality or quantity) if the good genetic potential of livestock does not support it. Furthermore, vice versa, if the livestock has good genetic potential, it will not be expressed optimally if the maximum environment and management do not support it. Thus, these two factors should receive equal attention in the maintenance of livestock commodities. Raising livestock with high genetic value accompanied by good management will certainly provide optimal results both in terms of production and business efficiency (Syukur, 2006).

Cows are livestock that is widely known by the general public, especially in rural areas. Cows in Indonesia are generally raised by farmers traditionally (from generation to

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generation), so that their development is fully in the midst of farmers (Utomo and Nur R., 2012). Beef cows are one of the resources for producing food in the form of meat, which has high economic value and is important for people's lives. A group of cattle can produce various kinds of needs, especially as a food ingredient in the form of meat and other by-products such as manure, leather, etc. (Sugeng, 2002). Furthermore, it is stated that based on their origins, cows are broadly classified into 3 groups, namely: Indicus bosses (Zebu cattle = humps), Taurus bosses (European cows), and Bos Sondaicus.

The need for animal protein from livestock in Indonesia is 5.4 g / capita/day. Based on these needs, Indonesians must consume 9.6 kg/cap/year of meat, 3.5 kg/cap/year of eggs, and 4.6 kg/capita/year of milk. The field's real conditions show that the Indonesian people can only meet animal protein consumption from livestock, an average of 3.47 g / capita/day (Harmadji, 1999). Conservation of genetic resources needs to be carried out with two considerations, namely the increasing demand for livestock products, especially in developing countries as the FAO predicts a 2-fold increase in demand for meat in the 30 years from 2000 to 2030 and the increasing demand for milk is more of two times, as well as the rapid reduction in almost all over the world of genetic resources (germplasm) (Subandriyo, 2006).

The implementation of AI (Artificial Insemination) in Indonesia began nationally in 1972. Through this AI activity, it is hoped that it will improve the genetic quality of local livestock breeds, so it is hoped that the cattle owned by the Indonesian people can gradually improve their genetic quality. In the development of national nurseries, the problems faced are how to increase the number of livestock breeds in good quality, the linkage and interdependence of breeding actors to provide livestock seeds in the number, type, and quality according to needs (Bahri, 2006). At the beginning of the AI implementation in Indonesia (1972 to 1973), using liquid semen (fresh). AI with fresh semen was felt to be very slow in development, so in 1973 with the help of frozen semen from the British and New Zealand governments, the use of frozen semen in straw (plastic straw) packaging was introduced. Since the use of frozen straws, AI development is very rapid to date, especially after the operation of BBAI in Lembang and Singosari as a producer of frozen straw, especially superior cow semen.

The main obstacle in implementing AI is the absence of cheap and effective field tools/flasks that can maintain the quality of frozen sperm in a straw when going to AI right. So far, inseminators always use flasks with liquid nitrogen in them to bring frozen straw to the field. So that costs are higher and less effective because liquid nitrogen is expensive and has limited availability. So it is necessary to create a tool with cheap cooling material but has the ability to maintain the quality of frozen sperm in a relatively long time.

II. Materials and Methods

A. *Materials and tools*

The research used frozen cement straws in the form of mini straw from the Pasundan cattle breed, which was produced by the BIB Lembang Beef Cattle Livestock, West Java. The research used an experimental method with a completely randomized design with a unidirectional pattern, namely ice and salt ratio with 3 replications. A total of 432 mini frozen cement straws, while the tools used include ice flasks, water jacket tubes, thermometers, microscopes, glass objects, cover glass, heating tables, and stationery.

B. *Method*

The research method used an experimental method with the basic completely randomized design with a unidirectional pattern, namely the ratio of ice cube vs. salt. The research will be repeated 3 times. *How it Works* : A total of 432 mini straws of frozen semen from Pasundan cattle were distributed in 48 flasks with a capacity of 750 ml made of stainless which were filled with nitrogen as T0 (control), T1: ice cubes + 30% salt, T2: ice cubes + 40% salt and T3: ice cubes + 50% salt. The straw is put into the water jacket in a large test tube then placed in the thermos standing up. Each flask contains 9 straws, which will be checked for motility (%) every 0, 2, 4, 6 hours of storage. Motility examination on the heating table using a microscope with a magnification of 10 x 40 times by looking at the spermatozoa object compared to spermatozoa that are not moving or moving but not progressive forward.

C. *Data Analysis*

To determine the difference in motility in the treatment of the ratio of ice and salt in a field flask, analysis of variance was carried out. If there is a difference, continue with the Duncan's Multiple Range Test (DMRT) difference test.

III. Result and Discussion

Post thawing motility

Motility is the movement of spermatozoa that can be used as a reference in assessing the quality of spermatozoa for artificial insemination (Bintara, 2011). The results of the complete examination of sperm motility of Pasundan cattle and BIB production in Lembang are presented in Table 1.

TABLE 1. Spermatozoa motility of Pasundan cattle

Treatment	Storage time (hours)				Average
	0	2	4	6	
T0	50.00 ±10.00	67.50 ± 2.50	58.33 ± 2.88	48.33 ± 2.88	56.04^{c} ± 9.13
T1	50.83 ± 10.10	30.00 ± 5.00	16.66 ± 2.88	11.66 ± 2.88	27.29^{a} ±16.63
T2	52.50 ± 2.50	30.00 ±17.32	18.33 ± 2.88	13.33 ± 5.77	28.54^{a} ± 17.66
T3	51.66 ± 9.46	40.83 ± 2.88	28.33 ± 5.20	24.16 ± 5.20	36.25^{b} ± 12.40
Average	51.25^{z} ± 7.42	42.08^{y} ±17.83	30.41^{x} ± 17.73	24.37^{x} ±24.31	

T0 for nitrogen, T1 for ice cube + 30% salt, T2 for ice cube + 40% salt, and T3 for ice cube + 50% salt
^{abc} different superscript in the same column show significant differences ($p<0.05$)
^{xyz} different superscript in the same line show significant differences ($p<0.05$)

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Based on these results, it shows that the media and storage time have a significant effect ($p<0.05$) on spermatozoa motility in T0 and T3 treatments, while T1 and T2 treatments are not significantly different ($p>0.05$). The use of storage media filled with liquid nitrogen had a higher motility rate ($p<0.05$) compared to a mixture of ice cubes and salt (T1, T2 and T3). This is because when the straw is stored with liquid nitrogen, the metabolism of spermatozoa cells can be said to stop, the metabolism of spermatozoa cells will increase along with the increase in temperature. Storage of semen in media with temperatures ranging from 3-4°C, metabolism is still running but very slowly, because it is far below the physiological temperature (38°C). The longer the storage time, the more use of nutrients between spermatozoa as a result of ongoing metabolism, this causes the availability of nutrients to be depleted. A large number of spermatozoa will accelerate the use of nutrients for the purposes of spermatozoa metabolism, so that nutrients will be quickly depleted and the production of ATP (adenosine triphosphate) in the mitochondria will be inhibited so that it can affect the motility of spermatozoa (Hayati, 2011).

The results of the research on the motility of the sperm of Pasundan cattle stored in a thermos with a storage medium of a mixture of salt and ice cubes showed that the best average motility that entered the SNI standard (Indonesian National Standard) was 40.83% in the T3 treatment (ice cubes + 50% salt) for a long time. two hours of storage, while the other motility averages were still below the SNI standard or their motility was below 40% except at zero hours of storage. This is because treatment using a mixture of salt and ice cubes causes cold shock. Changes in temperature that occurred, namely from -196°C (liquid nitrogen temperature) to the temperature of ice cubes and salt treatment in the thermos, namely -15°C, made most of the spermatoza die and their motility decreased. According to Sonjaya et al., (2005) stated that the biggest factors that affect the decrease in sperm quality due to cold shock are temperature and pH.

Garner and Hafez (2000) stated that the motility of bovine spermatozoa ranged from 40-75%. Differences in spermatozoa motility can be caused by age in cattle, 1.5 years old has lower motility than 2 years old, this is because in 2 year old cows the primary and secondary reproductive organs are optimal (Azzahra et al., 2016). Meanwhile, according to Herdis (2005) states that the motility of spermatozoa is influenced by differences in the breed of livestock and the time of examination. Another factor that can affect motility is feed (Zulfan, 2008).

Viability of spermatozoa

The results of the examination of spermatozoa viability of Pasundan cattle and BIB production in Lembang are presented in full in Table 2.

TABLE 2. Viability of spermatozoa (%)

Treatment	Storage time (hours)				Average
	0	2	4	6	
T0	$79.33^{h}\pm2.56$	$74.05^{gh}\pm6.05$	$71.24^{gh}\pm12.78$	$67.36^{gh}\pm10.31$	$72.99^{e}\pm8.81$
T1	$72.16^{gh}\pm5.48$	$58.05^{gh}\pm20.56$	$52.42^{g}\pm24.0$	$26.35^{f}\pm12.72$	$52.24^{d}\pm22.73$
T2	$71.50^{gh}\pm2.17$	$67.66^{gh}\pm8.31$	$20.17^{f}\pm11.82$	$20.17^{f}\pm11.82$	$47.64^{d}\pm25.42$
T3	$58.67^{gh}\pm4.73$	$65.07^{gh}\pm11.14$	$29.83^{f}\pm6.11$	$19.91^{f}\pm9.25$	$43.37^{d}\pm21.01$
Average	$70.41^{c}\pm8.48$	$66.21^{c}\pm12.43$	$46.18^{b}\pm22.81$	$33.44^{a}\pm22.69$	

T0 for nitrogen, T1 for ice cube + 30% salt, T2 for ice cube + 40% salt, and T3 for ice cube + 50% salt
^{abc} different superscript in the same line show significant differences ($p<0.05$)
^{de} different superscript in the same column show significant differences ($p<0.05$)
^{fgh} different superscript in the same line and column show significant differences ($p<0.05$)

Based on these results, it shows that the media and storage time have a significant effect ($p<0.05$) on live spermatozoa. The use of liquid nitrogen (T0) was higher ($p<0.05$) compared to a mixture of ice cubes and salt (T1, T2 and T3). However, the use of ice cubes and salt at all levels (T1, T2 and T3) showed no significant difference ($p<0.05$). Viability of spermatozoa decreased by 29.62%. This percentage decrease occurred when using ice cubes and salt media (T1, T2 and T3). The percentage of spermatoza viability decreased due to damage to the plasma membrane and acrosome membrane due to the influence of cold shock. The temperature of the frozen cement storage media used in this study was different, namely M0 (-196°C). This is in accordance with Septiani et al. (2017) that the metabolic rate and motility of spermatozoa will decrease at a temperature of 3 to 5°C. Viability is the vitality of spermatozoa as an indicator of spermatozoa quality. The viability of diluted frozen semen has at least 60 to 75% live spermatozoa (Garner and Hafez, 2000).

The storage time of 0 and 2 hours was higher ($p<0.05$) than 4 and 6 hours. However, the storage time of 4 hours tended to be better ($p<0.05$) than 6 hours. Based on the results of the study, the length of storage time decreased the viability of spermatozoa by 36.70%. The longer the storage time, the decrease in viability is also high. Spermatozoa quality is said to be good if it has a high number of live spermatozoa and <15% dead spermatozoa (Bintara, 2011).

The results of the analysis of variance showed that there was a significant interaction between the media and storage time ($p<0.05$). This shows that the media and the length of storage time affect each other's viability. The use of M0 with a shelf-life of 0 hours showed the highest viability compared to other treatments and T3 with the lowest curing time of 6 hours ($p<0.05$). The use of T1 with a storage time of 2 hours tends to be better than T2 and T3 although it is not significant.

Likewise with the long storage time of 6 hours. The percentage of viable spermatozoa is determined by the intact plasma membrane. The plasma membrane functions to protect

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spermatozoa organelles and transport electrolytes for spermatozoa metabolism. Damaged plasma membrane can affect the physiological function and metabolism of spermatozoa, causing spermatozoa to die. Intact plasma membrane has a correlation with spermatozoa motility, the more intact spermatozoa plasma membrane, high motile spermatozoa (Azzahra et al., 2016). In this study, the decrease in viability occurred because the spermatozoa experienced cell damage, namely changing the structure of the plasma membrane phospholipids and disrupting the permeability function of the cell membrane. If there is damage to the membrane, the metabolic process will be disrupted, the synthesis of Adenosine Tri Phosphate (ATP) does not work well and results in a decrease in the viability of spermatozoa.

Wave motion

Spermatozoa mass movement is the active movement of spermatozoa by showing undulating and massive mass movements. The mass movement scores consist of very good (3/+++), good (2/++), moderate (1/+), and bad (-) (Tambing et al., 2001). The results of the examination of the mass movement of the spermatozoa of Pasundan cattle and BIB production in Lembang are presented in Table 3.

TABLE 3. Mas motion of Pasundan cattle spermatozoa

Treatment	Storage time (hours)				Average
	0	2	4	6	
T0	2.00 ± 0.00	2.16 ± 0.28	1.83 ± 0.28	1.83 ± 0.28	1.95 ± 0.25[b]
T1	2.00 ± 0.00	1.16 ± 0.28	1.00 ± 0.00	1.16 ± 0.28	1.33 ± 0.44[a]
T2	2.33 ± 0.28	1.33 ± 0.28	1.16 ± 0.28	1.33 ± 0.28	1.54 ± 0.54[a]
T3	2.00 ± 0.50	1.66 ± 0.57	1.33 ± 0.28	1.16 ± 0.28	1.54 ± 0.49[a]
Average	2.08[y] ± 0.28	1.58[x] ± 0.51	1.33[x]± 0.38	1.37[x]± 0.37	

T0 for nitrogen, T1 for ice cube + 30% salt, T2 for ice cube + 40% salt, and T3 for ice cube + 50% salt
[ab] different superscript in the same line show significant differences ($p<0.05$)
[xy] different superscript in the same column show significant differences ($p<0.05$)

The results showed that the average mass movement of spermatozoa ranged between good and sufficient categories, this could occur because treatment using salt and ice caused cold shock. Extreme temperature changes that occur, namely from a temperature of -196^{0}C (liquid nitrogen temperature) to the temperature of the ice cube and salt treatment in the thermos, which is -15^{0}C, make most of the spermatoza dead. The biggest exogenous factors that affect the decrease in sperm quality due to cold shock are temperature and pH (Sonjaya et al., 2005).

Cold shock that occurs due to changes in extreme temperatures and storage time in the treatment causes the metabolism of spermatozoa to run very quickly, causing the energy for locomotion to decrease and ultimately causing the spermatozoa to die. Spermatozoa will use energy for locomotion produced by accessory glands (Azzahra et al., 2016). Accessory glands will secrete plasma semen by producing several kinds of nutrients such as glucose, fructose, sucrose, citric acid, protein, potassium, sorbitol, insitol and glycerylphosphoryl-choline (GPC) which function as energy substrates for spermatozoa including sperm motility (Sujoko, 2009).

Cold shock that occurs also increases the production of lactic acid in the sperm to be high as a result of the rapid metabolic process. The high content of lactic acid in sperm will result in increased damage to the membrane that affects metabolism and the energy produced. The high amount of lactic acid will affect the increase in the osmotic pressure of the semen plasma thereby reducing the permeability of the spermatozoa membrane and increasing membrane damage. Damage to the spermatozoa membrane will affect the metabolic process and decrease the formation of energy. The reduced energy will greatly affect the activity and mobility of spermatozoa (Samsudewa et al., 2006). This is what causes the large number of dead spermatozoa. It can be concluded that the storage of spermatozoa in a thermos with salt and ice treatment was not able to maintain the mass movement of spermatozoa significantly.

IV. Conclusion

T3 shown the best results for the variable motility, viability and wave motion.

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References

[1]. Arifin, J., Dudung Mulliadi, 2014. The Potencial Rancah Cattle in West Java. Department of Animal Husbandri of West Java. Bandung
[2]. Arifin, J, Sri Bandiati K, Endang Y. S, Unang Y, Asep A,Indrijani, Sulasmi. 2015. Gene Distribution, Population Balance and Effective Population Size of Pasundan Cattle Post Migration in Majalengka. *Journal of Animal Science*, Vol 15, No 2
[3]. Azzahra, F.Y., E.T. Setiatin dan D. Samsudewa. 2016. Evaluation of Sperm Motility and Viability of Fresh Semen of Kebumen PO Bulls. *Journal of Animal Science Indonesia* Vol. 11 No. 2.
[4]. Bahri, S., 2006. National Breeding Policy in Relation to the Utilization of Livestock Germplasm. Director of Breeding of the Directorate General of Manpower. *National Meeting on Preservation and Development of Indonesian Germplasm*, Faculty of Animal Science Universitas Gadjah Mada.
[5]. Bintara, S. 2011. X:Y Ratio and Sperm Quality in Local Goats and Ettawa Crosses. Fakulty of Animal Science, Universitas Gadjah Mada. Yogyakarta. *Journal of Animal Science*, 9(2):65-71.
[6]. Dinas Peternakan Provinsi Jawa Barat. 2014. Sustainable Local Livestock Genetic Resources Management Program in West Java. Bandung. West Java.
[7]. Djanuar, R., Haryati, Rachmawati, T R Tagama., 1988. IB Basics in cattle. Lab-Fisrep, Faculty of Animal Science, Jenderal Soedirman University. Purwokero.

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[8]. Feradis., 2010. Reproductive Biotechnology in Livestock. Publisher Alfabeta, Bandung.
[9]. Garner D.L. and E.S.E. Hafez. 2000. Spermatozoa and Seminal Plasma. In: E. S. E.Hafez (Ed.). Reproduction in Farm Animal. 7th. ed. Lippomcott Wiliams and wilkins. Philadelphia. 96-106.
[10]. Hafez, ESE., 1987. *Reproduction in Farm Animal*, 4 th ed. Lea and Febiger, Philadelphia.
[11]. Hayati A, 2011. *Spermatology*. Surabaya: Publishing and Printing Center Universitas Airlangga. Surabaya.
[12]. Hilmia N. 2013. Phenotype Characterization and Genetic Potential and Their Relationship to Productivity and Quality of Local Beef Ciamis West Java. *Thesis*. Bogor. IPB University.
[13]. Menteri Pertanian RI. 2014. Decree of the Minister of Agriculture Number 1051/Kpts/SR.120/10/2014 concerning Establishment of Clumps of Pasundan Cows in West Java. Minister of Agriculture Republic of Indonesia. Jakarta
[14]. Naufal, F, Endang Yuni Setiawati dan Nono Suwarno, 2016. "Qualitative Characteristics of Pasundan Cattle in People's Farms" (http: // Jurnal. unpad. Ac .id / ejurnal / article / download / 10135 / 456) Acces date: 6 Oktober 2017.
[15]. Partodihardjo, S., 1992. Animal Reprodutive Science. Publisher Mutiara, Bandung.
[16]. Salisbury, G.W. dan N. L. Van D emark. 1985. Reproductive Physiology and Artificial Insemination in Cattle. Gadjah Mada University Press. Yogyakarta.
[17]. Samsudewa, D. dan E. Purbowati. 2006. Size of male local sheep reproductive organs at different ages. In: National Seminar on Animal Husbandry and Veterinary Technology. 413-418.
[18]. Septiani, D., E. M. Effendi, Moerfiah. 2017. Storage of spermatozoa at room temperature reservation and various semen diluents on the survival of spermatozoa. Ekologia, 17 (2): 18-23.
[19]. Sonjaya. H, Sutomo dan Hastuti. 2005. Effect of Addition of Calcium IonopHore on Spermatozoa Quality of Boer Goats from Sexing. J. Science & Technology 5 (2).
[20]. Sujoko, H, M. H. A. Setiadi dan A. Boediono. 2009. Spermatozoa selection of arrowroot sheep using Percoll density gradient centrifugation method. Journal of Veteriner. 10 (3) : 125-132.
[21]. Tambing, S.N, M. Gazali. dan B. Purwantara. 2001. Empowerment of Artificial Insemination Technology in Goats. Wartazoa Vol. 11, No.1.
[22]. Toelihere, M. R. 1985. Artificial Insemination in Cattle. Angkasa. Bandung.
[23]. Utomo, S. dan N. Rasminati, 2012. Areas. Proceedings of the National Seminar on Optimizing Research and Service in Building Character People. LPPM Universitas Negeri Yogyakarta. Page 755-764.
[24]. Utomo, S. dan N. Rasminati, 2013. The Effect of Differences in Altitude on the Achievement of Artificial Insemination Results in Cattle. *Journal of Animal Sciene*, Departement of Animal Science, Universitas Sebelas Maret. Vol 11, No 1, Maret 2013. Hal : 34-42
[25]. Utomo,S., 2004. Achievement of reproduction level of local cattle and sheep at farmer level in Bantul district, Independent research. Animal Husbandry Study Program, Faculty of Agroindustry. Universitas Mercu Buana Yogyakarta.

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