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The Effect of Thawing Duration on the Post Thawing Quality of Bali Cattle's Frozen Semen and Conseption Rate in Smallholder Farms of East Lombok Regency

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ABSTRACT

This research aimed to identify the effect of the length of thawing time on the quality of Bali cattle postthaw semen and to investigate the resulting pregnancy rate. This research was conducted at Wanasaba Village, specifically Tanaq Mira Village, Wanasaba District, East Lombok Regency. Five samples of Bali cattle semen were collected for insemination from each of three different farmer groups. The semen sample from the remaining frozen semen inseminated by the inseminator was used in this research. Tanaq Mira Village's inseminator performed thawing during the trip from the artificial insemination (AI) station to the farmer group's location. This research observed the microscopic quality of the postthaw frozen semen covering motility, viability, and abnormality. Moreover, the pregnancy rate on AI acceptors using the non return rate (NRR) parameter or the number of female parents who returned to estrus after being inseminated were observed. The observation was conducted on three farmer groups with different distances and lengths of thawing time. Each farmer group was observed five times. The collected data were then analysed by using one-way analysis of variance (ANOVA). The research indicated that the distance of the farmer group's location significantly affected ($P<0,01$) the sperm motility and viability. However, it did not significantly affect ($P>0,05$) the sperm abnormalities. The Sapeng farmer group had the highest NRR, as much as 80%. According to the NRR score, it can be concluded that the thawing process using the thermos within less than 10 minutes resulted in the best yield.

Keywords: Abnormality, Bali cattle, Motility, Non return rate, Thawing, Viability

Introduction

The meat consumption of Indonesian people, especially beef consumption, has increased from year to year. In 2018, the beef consumption of the Indonesian people increased compared to the previous years. In 2012 and 2014, beef consumption was 0.261 kg/capita/year. In 2016 and 2017, it increased to 0.417 kg/capita/year (Kementerian Pertanian, 2018). However, the increase in beef consumption is not followed by an adequate national meat supply, thus some of the community's needs are fulfilled through imports. Indonesia has various local livestock resources that produce meat, such as goats, sheep, chickens, horses, buffalo, and cattle. Cattle, as one of the highest meat contributors, are livestock commodities that are generally raised and consumed in almost all parts of Indonesia. Indonesia has many breeds of local beef cattle that can be consumed, one of which is Bali cattle.

Bali cattle are one of Indonesia's local cattle that have spread to almost all area of Indonesia. Bali cattle are the result of the domestication of wild banteng (*Bibos Banteng Syn Bos Sondaicus*), which is now claimed to be local Indonesian cattle (Purwanti and Harry, 2006). Bali cattle are ideal beef cattle to be raised in Indonesia because of their high adaptability. Increasing the population of Bali cattle is necessary to fulfil the meat needs of the Indonesian people. One of the efforts to achieve this goal is by applying artificial insemination technology.

Artificial insemination (AI), often called mating injection, is a method of livestock breeding carried out by inseminators through depositing semen into the corpus uteri. Artificial insemination is the appropriate way to increase the Bali cattle population. The AI technology can increase the cattle population because male cattle can produce approximately 20,000 offspring in a year compared to natural mating, which only results in 40 offspring (Purwati and Harry, 2006). In

addition, AI is also a technology that has been widely applied by breeders in Indonesia, both industrial-scale breeders and smallholder farmers in groups, because the price is relatively cheap when compared to other technologies such as embryo transfer (ET). However, until now, the application of AI in smallholder farms, especially in West Nusa Tenggara (Nusa Tenggara Barat; NTB), has not been optimal.

The number of AI acceptors in the NTB is 43,333 heads, with as many as 23,872 (55.08%) AI births (NTB in Numbers, 2018). The AI success rate of 55.08% should be improved. Many factors influence the success of AI, such as motivation to raise livestock, maintenance systems, breeders' knowledge, AI acceptor cows, inseminators, and semen quality. This research evaluates the microscopic quality of Bali cattle semen after thawing, which is ready to be inseminated. Utomo and Boquifai (2010) reported that the average motility of frozen semen after thawing at 37°C for 30 seconds was 47.24%, which was higher than the average motility of frozen semen after thawing at 5°C (41.12%) and 27°C (45.52%). Thawing carried out by the inseminator of Wanasaba Village is different from the set conditions. The thawing method affects semen quality. This research was conducted to analyse the effect of the thawing method on semen quality and the success of artificial insemination. This study also aimed to analyze the effect of thawing duration on the postthawing quality of Bali cattle semen and to analyze the resulting pregnancy rates. The distance can affect the quality of postthaw semen because the thawing process is carried out during the trip, so the farther the distance, the longer the thawing process will take and can affect the quality of semen. To date, no research has been conducted on this topic in NTB, as the conditions of the farms and the locations of the farmer groups are at different altitudes. As a result, it can affect the inseminator's work in performing the insemination. This research is expected to be a consideration and evaluation to improve the quality of AI applications, especially in the East Lombok Regency.

Materials and Methods

Place and time

This research was conducted from March to May 2021 in Tanaq Mira Village, Wanasaba District, East Lombok Regency, NTB. Wanasaba District, especially Wanasaba Lauk Sub-District, Tanaq Mira Village, is ± 64 km from the city of Mataram. The area of Wanasaba District is 55.89 km^2 and consists of 14 villages with an altitude ranging from 144 – 522 masl with relatively low rainfall from July October (Badan Pusat Statistik Lombok Timur, 2018). The equipment used in this research is a microscope. The microscope used is a binocular microscope type XS-201, the cover glass used is 20 × 20 Menzel Glaser brand, the object-glass used is sail brand, dropper pipette, thermometer, counter, bunsen burner, and a camera. The main ingredients used were cows of productive age raised by farmers in Tanaq Mira Village, Bali cattle straw from the Lelede Regional Artificial Insemination Center, West Nusa Tenggara, and 2% eosin solution as a dye.

Experimental design

This research applied a completely randomized design (CRD) with four treatment levels. The first treatment level was the control (P0), which was thawing at 37°C for 30 seconds and carried out directly without farmer groups. The second treatment level (P1) involved thawing at ≤5 minutes and was observed in the Sapeng farmer group. The third treatment level (P2) involved thawing at >5 minutes to 10 minutes and was observed in the Tunas Ridha Ilahi farmer group. The fourth level of treatment was thawing at more than 10 minutes and was observed in the Sumber Rezeki farmer group. At each treatment level, five observations will be made. After observing each treatment level, the data were collected.

Population and sample

This research was conducted on three farmer groups: the Sapeng farmer group, the Tunas Ridha Ilahi farmer group, and the Sumber Rezeki farmer group. These three farmer groups are members of the Ridha Ilahi Smallholder Farm Center (SFC). Ridha Ilahi SFC guards 21 farmer groups spread across various villages in Wanasaba District, including those three groups.

This research was conducted on three farmer groups who were selected based on the distance and travel time from the AI station to the group, as presented in Table 2. At least 5 AI acceptors in each group were observed. In this research, we used the judgment sampling method. Judgement sampling is a sampling method where the researcher selects a sample from the population based on an assessment because the researcher believes that the selected sample represents the population and corresponds to the population being studied.

Thawing carried out by inseminators in Wanasaba District, especially Tanaq Mira Village, was different from the standard that had been set, which is thawing at 37°C for 30 seconds. The inseminator in Tanaq Mira Dusun performed the thawing process during the trip from the AI station to the farmer group's location. The straw that had just been removed from the liquid nitrogen container was put into thermos filled with tap water at a temperature of 27°C to 29°C, and the thawing process took place during the trip to the farmer group. After the inseminator arrived at the farmer group's location, the insemination was carried out as soon as possible.

The variables observed in this research were motility, viability, and abnormalities in frozen semen of postthawed Bali cattle, and we observed the success of AI by looking at the number of pregnancies resulting.

Motility. According to Wahyuningsih *et al.* (2013), motility is the movement of individual sperm that is used as a measure of the sperm's ability to fertilize an ovum. Determination of the percentage of spermatozoa motility can be performed by dripping semen on a clean object glass and covering it with a cover glass. Individual motility was examined subjectively on an object glass that was then covered with a cover glass and examined under a microscope with a magnification of 10 × 40. In the assessment stage, one drop of the semen sample is dripped on a glass object and covered with a cover glass and then placed on a heating board at a temperature of 37°C, which is directly attached to the microscope. Assessment of motile spermatozoa was carried out in three different fields of view with a microscope with a magnification of 400 times for each semen sample. The mean of three consecutive estimates is recorded as the final motility score.

Viability. Viability is the vitality of spermatozoa. Examination of spermatozoa viability can be used as an indicator of the structural integrity of spermatozoa membranes (Prastika *et al.*, 2018). The calculation of the percentage of viability was carried out by dripping one drop of thawed semen on a clean object glass, then dripping with eosin dye on top of the semen and mixed evenly using a sterile glass rod. A thin smear preparation was immediately dried over a flame and then observed under a microscope at a magnification of 400 times. Dead spermatozoa heads will absorb the red color, while those that are still alive will not absorb the dye.

$$\text{Viability (\%)} = \frac{number\ of\ live\ spermatozoa}{total\ number\ of\ spermatozoa} x100$$

Abnormality. Abnormality is one of the indicators in determining the quality of spermatozoa, as abnormal cell structure can cause disturbances and obstacles at the time of fertilization, further causing low rates of implantation and pregnancy. In addition to the grouping of primary and secondary abnormalities (Afiati *et al.*, 2015), sperm morphology abnormalities are abnormal sperm shapes that occur in the head and tail (Andrefani *et al.*, 2019). Spermatozoa abnormalities were observed by making smear preparations on an object glass from one drop of sperm mixed with one drop of eosin dye. Observations were made under a microscope with a magnification of 400 times. Spermatozoa with abnormal morphology can be counted by the formula:

$$\text{Abnormality (\%)} = \frac{number\ of\ abnormal\ spermatozoa}{total\ number\ of\ spermatozoa} x100$$

Pregnancy rate. Observation of pregnancy rate was measured by using the non return rate parameter. Non return rate (NRR) is the number of female cattle that do not return to estrus on the 21st day after insemination, with the assumption that female cattle that are not in estrus again are pregnant (Saili *et al.*, 2017).

The formula for calculating the NRR according to Iswoyo and Widiyaningrum (2008) is as follows:

$$\text{NRR (\%)} = \frac{\Sigma\ AI\ cows - \Sigma\ Repeat\ AI\ cows}{\Sigma\ AI\ cows} x100$$

The data was analyzed using one-way analysis of variance (ANOVA) to determine differences in motility, viability, and abnormalities in each group. If there was a significant difference, then it was continued with Duncan's multiple distance test.

Results and Discussion

The results of observations of the microscopic quality of frozen semen of Bali cattle after thawing in three groups of livestock in Wanasaba Lauq village resulted in various yields, as presented in Table 3.

The distance from the AI station to different farms provided a different average thawing time length for each farmer group. The Sapeng group with a short distance had an average thawing time of 2 minutes 30 seconds; the Tunas Ridha Ilahi group with a medium distance had an average thawing time of 7 minutes 5 seconds; and the Sumber Rezeki group with a long distance had an average thawing time of 13 minutes 40 seconds.

Post-thawing motility

Motility or individual movement of sperm is an assessment used as a measure of the ability to fertilize an ovum. Motility assessment is carried

Table 1. Cattle population

Farmer group	Number of cattle (head)	Number of parent (head)	Number of member (people)
Sapeng	59	41	10
Tunas Ridha Ilahi	46	35	15
Sumber Rezeki	48	38	11
Total	153	114	36

Table 2. Farmer groups at Tanaq Mira Wanasaba Village

Farmer group	Distance	Thawing time lenght (minute)
Sapeng	Close	<5
Tunas Ridha Ilahi	Medium	5-10
Sumber Rezeki	Far	>10

out by assessing the movement of spermatozoa individually, either the speed or the ratio between those that are actively moving progressively with other spermatozoa movements (Arifiantini, 2012). Spermatozoa motility is also used as a reference for male fertility, as the progressive movement of spermatozoa is expected to accelerate the encounter with the ovum for the fertilization process in the female reproductive tract (Mahfud *et al.*, 2019). Spermatozoa motility assessment can be performed on fresh semen or frozen postthaw semen.

Post-thawing motility is an examination of the motility or movement of individual spermatozoa after thawing before insemination, with the postpost-thawing motility (PTM) value of frozen semen not less than 40% following SNI 4869-1: 2017 (Fazrien *et al.*, 2020). The National Standardization Agency has determined that frozen semen thawing must be carried out in 37°C water for 30 seconds to maintain sperm quality. Too fast-frozen semen thawing can cause ice crystals to not melt completely, thereby inhibiting the active movement of spermatozoa cells. However, too long thawing can reduce the quality of spermatozoa (Adnyani *et al.*, 2018). The following are the results of the postthawing motility assessment of frozen semen of Bali cattle in East Lombok at different thawing times.

The inseminators of Tanaq Mira Village, Wanasaba Lauq Subdistrict, often carry out the thawing process by using tap water because it is considered more practical than providing warm water. The thawing performed by the inseminator of the Wanasaba Lauq Subdistrict showed a very significant difference ($P<0.01$) in sperm motility after thawing. Thawing for more than 10 minutes was significantly different from thawing for 5 to 10 minutes, thawing for less than 5 minutes, and thawing for 30 seconds. A thawing duration of more than 10 minutes showed a motility value that was far less than the quality of frozen semen suitable for insemination. The longer the thawing duration was, the lower the motility value. This occurrence is in line with the research by Samsudewa and Suryawijaya (2008) that thawing with ice water for 30 minutes and 60 minutes in Simental cattle shows a decreased motility value as well as in Limousin, which experienced a decrease in individual motility value to a quality that can no longer be used for AI (<40%). Salim *et al.* (2012) stated that it was because the thawing temperature was too low in this treatment and was not under the physiological conditions of spermatozoa movement that the motility of the spermatozoa was low. This also indicates that the lower the thawing temperature is, the greater the decrease in individual motility. This statement is supported by other research from Andrefani *et al.* (2019), who confirmed that thawing performed at a temperature of 29°C for 15 seconds and 30 seconds showed a higher percentage of motility than thawing for 45 and 50 seconds. It is suspected that in the 15- and 30-second treatments, the metabolism of spermatozoa ran perfectly because it was under the normal physiological temperature in Bali cattle. At physiological temperatures, the activity of the enzymatic reactions that took place during cell metabolism was optimal. Salim *et al.* (2012) explained that the decrease in the quality of spermatozoa is due to the increased metabolic activity of spermatozoa that massively occurs and that there is an increase in lactic acid production so that the concentration of toxic lactic acid increases, resulting in low sperm motility or death. A decrease in the quality of spermatozoa after thawing will reduce the ability to fertilize and affect embryonic development (Khalil *et al.*, 2018).

Thawing in the control treatment or thawing carried out for 30 seconds at 37°C-38°C showed the highest motility value. Utomo and Boquifai (2010) explain that at 37°C, the movement of individual sperm is achieved optimally because the energy produced by metabolism is also maximum, so thawing in water with a temperature of 37°C - 38°C will result in better motility of spermatozoa than thawing at lower temperatures. Thawing with 37°C water can support semen to pass its critical period quickly because the temperature is the same as the body temperature of livestock.

The decrease in sperm quality occurs at every stage of semen processing, even since the freezing process. Hapsari *et al.* (2018) state that during the freezing process, approximately 50% of spermatozoa will die. Mostly, there will be a decrease in motility and viability of spermatozoa. Various treatments can cause spermatozoa to die, such as the cold shock effect, but in general, approximately 50% - 60% of spermatozoa will be able to survive until the thawing stage with appropriate procedures (Gangawar *et al.*, 2016). According to Fraser *et al.* (2014), prolonged storage affects the motility, mitochondrial function, and plasma membrane integrity of spermatozoa. Removing the mini straw from the container will automatically increase the temperature (natural thawing) from freezing temperature to room temperature (Utomo and Boquifai, 2010) state that semen thawing should be inseminated in no more than 5 minutes.

Post-thaw abnormality

Abnormality is one of the indicators in determining the quality of spermatozoa. This is because abnormal cell structure can cause disturbances and obstacles at the time of fertilization, further causing low rates of implantation and pregnancy. Abnormality is divided into primary and secondary abnormalities (Afiati *et al.*, 2015). Regardless of primary or secondary abnormalities, sperm can fertilize an ovum if the sperm abnormality is less than 20% (Soeparna and Nurcholidah, 2014). The following is the average abnormality in postthaw frozen semen of Bali cattle at different thawing time lengths and distances.

Thawing time length and distance had no significant effect ($P>0.05$) on abnormality values

Table 3. The results of the microscopic quality evaluation of frozen semen from Bali cattle

Variable	Distance			
	Control	Close (Sapeng)	Medium (Ridha Ilahi)	Far (Sumber Rezeki)
Motility (%)**	52.80±8.70[b]	52.00±12.49[b]	36.20±14.77[b]	19.80±10.49[a]
Viability (%)**	61.88±6.83[b]	63.15±11.73[b]	58.47±10.35[b]	38.13±8.64[a]
Abnormality (%)	9.98±6.09	11.14±3.29	8.26±4.72	8.19±4.04

[a,b] Different superscripts in the same row show significant (P<0.05) and very significant differences (P<0,01).
** Very significant difference.

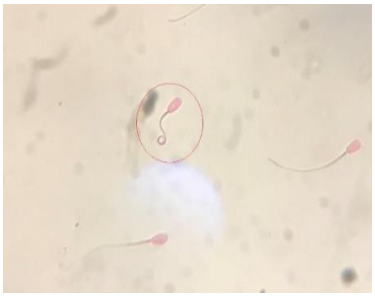

Figure 1. Secondary Abnormality (*Coiled Double Tail*).

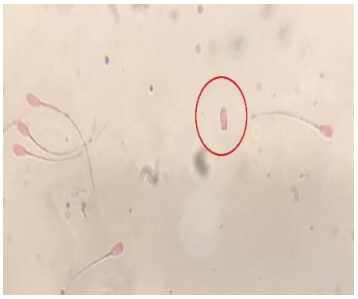

Figure 2. Secondary Abnormality (*Translocating Cytoplasmic Droplets*).

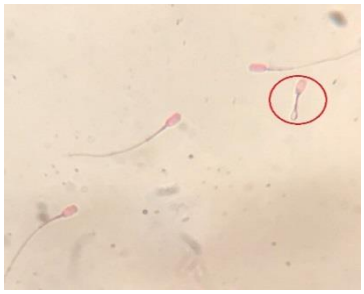

Figure 3. Primary Abnormality (*Decapitated).*

in frozen semen from Bali cattle after thawing. This result indicated that the temperature and duration of thawing at all treatment levels did not cause the spermatozoa to become abnormal. This lack of influence may be due to the temperature and duration of thawing carried out by the inseminator of Tanaq Mira Village not applying extreme mechanical pressure that leads to abnormalities of spermatozoa. The abnormality value in each group was still feasible for insemination because it was less than 20%. Farmer groups with short distances or thawing durations of fewer than 5 minutes had the highest abnormality values. According to Adnyani *et al.* (2018), spermatozoa abnormalities may occur due to the freezing process, the thawing process, and during the microscopic evaluation process, such as the manufacture of smear preparations. Abnormalities occur presumably because the spermatozoa cell membrane is damaged and causes an unstable cell membrane due to semen processing starting from storage, dilution, storage, and handling after storage, such as making preparations for sperm quality observation (Mahfud *et al.*, 2019).

According to Utami and Tarsisius (2014), morphological abnormalities of spermatozoa can occur in primary, secondary, or tertiary stages. Primary spermatozoa abnormalities include a head that is too small (microcephalic), a head that is too large (macrocephalic), a wide, elongated, multiple heads, multiple bodies or tails, and a coiled center (tail coiled). Secondary abnormalities include a bent tail, proximal droplet, and simple bent tail. According to Salim *et al.* (2012), one of the characteristics of spermatozoa with tertiary abnormalities is a severed tail or head. This spermatozoa abnormality may occur due to the influence of the thawing treatment and the preparation process.

Abnormalities that often occur in frozen semen of Bali cattle in Tanaq Mira Village are circular and severed tails or commonly called simple bent tails that can cause sperm to be unable to move progressively forward and only move in circles in place so that the spermatozoa cannot fertilize the oocyte (Putranti, 2010). 2016). Some abnormalities often occur in the head. Spermatozoa with severed heads and tails cannot fertilize the oocyte because there is no tail to move towards the ovum. This opinion is in line with Ariantie *et al.* (2013), who state that frozen semen with a fairly high percentage of abnormalities tends to have low fertility because it relates to the ability to fertilize or maintain embryonic development.

These characteristics indicate that the spermatozoa have secondary and tertiary abnormalities. According to Salim (2012), one of the characteristics of spermatozoa with tertiary abnormalities is a severed tail or head. Yulnawati *et al.* (2009) argue that tertiary abnormalities may occur due to the manufacture of smear preparations that cause the head or tail of the spermatozoa to break off. The tertiary abnormality condition was not caused by the thawing process or the previous process but was caused by the process of making the preparations for the review. This statement is confirmed by Arifiantini and Purwantara (2010), who state that drying too fast and heating at high temperatures in the process of making preparations for review can affect the percentage of spermatozoa abnormalities. Yulnawati and Setiadi (2005) also state that the presence of toxic substances both from dead spermatozoa and from substances contained in diluents that have been oxidized due to storage can cause high levels of free radicals that can damage the integrity of the spermatozoa plasma membrane.

Post-thaw viability

Viability is the vitality of spermatozoa, which is known by observing the number of live and dead spermatozoa by staining with eosin negrosin. Dead spermatozoa will absorb the eosin solution to become pink. The absorption of the color is due to damage to the cell membrane, possibly due to semen processing (Mahfud *et al.*, 2019). The percentage of viable spermatozoa is determined by the intact plasma membrane. The calculation of the percentage of viable spermatozoa in this research was based on 2% eosin staining. Dead spermatozoa are indicated by stained spermatozoa heads, while live spermatozoa are indicated by nonabsorbent colouration (Adnyani *et al.*, 2018). According to Irsan (2015), spermatozoa that are still alive cannot penetrate the spermatozoa cells with 2% eosin dye. This is due to the impermeable spermatozoa membranes to eosin dye, while dead spermatozoa have damaged membranes so that the eosin dye can enter the spermatozoa cells.

The thawing time length had a very significant effect (P<0.01) on the sperm viability value of Bali cattle. Thawing at more than 10 minutes showed a very significant difference from other treatment levels, including the control treatment level. Based on the results of the microscopic evaluation, it can be seen that the value of sperm viability decreased with increasing thawing time. Jaelani *et al.* (2014) reported that the viability value of Bali cattle spermatozoa that were thawed with ice water at 3°C decreased with increasing thawing time. The value of viability with thawing for 30 minutes was 56.60% and decreased in thawing for 120 minutes to 46.00%. Viability values in medium and close farmer groups can be categorized as good and can be used for insemination activities because their viability is more than 40%, corresponding to the opinion of Witarsa (2001), who also states that postthawing spermatozoa survival is at least 40%. If less than 40%, then the frozen semen is not suitable for insemination. In addition, the percentage is said to be good because it is still above the percentage of motility. According to Arifiantini *et al.* (2006), the percentage of live spermatozoa must be above the percentage of motility. Frozen semen is regarded as quite good because it is still able to maintain the quality of spermatozoa, which is higher than motility.

Ansari *et al.* (2010) explained that there is a percentage decrease in frozen semen viability with a longer thawing duration. This may be due to the inappropriate thawing temperature and duration resulting in spermatozoa membrane damage caused by heat stress and contact with oxygen both when removed from the container and during insemination. Arifiantini and Purwantara (2010) also explain that a decrease in the viability percentage is possible. If the temperature and thawing time length are below optimal, the spermatozoa will experience cold shock. The decrease in the value of viability after thawing may be due to changes in temperature that cause increasing changes in cell metabolism, resulting in lactic acid accumulation and further causing lipid peroxidation in the plasma membrane (Gordon, 2017).

Non return rate

The non return rate (NRR) is one of the parameters that can be used to observe the pregnancy rate in AI cows. Observations were made by calculating the percentage of the number of females that did not show estrus again after accepting AI with observations 21 days after AI. The AI acceptors used as samples in this study were generally heifers because Bali cattle straw was the farmers' last choice, so they were inseminated in heifers. The principle of the NRR (non return rate) is to observe estrus between post-AI estrous cycles (Lopulalan *et al.*, 2018). The following are the results of observing the success of artificial insemination by assessing the non return rate in the first estrous cycle after insemination using frozen semen of Bali cattle in Tanaq Mira Village, Wanasaba District.

Farmer groups with different distances significantly affected (P<0.05) the non return rate (NRR). Further test results showed that the Sapeng farmer group had the highest non return rate (NRR) (80%) compared to the Tunas Ridha Ilahi group and the Sumber Rezeki group. This score can be regarded as good because it is in line with Jalius (2011), who reports that the better the sperm quality is, the greater the success of artificial insemination. It is based on the best NRR value of 84% in Bali cattle in Bengkulu city, which is due to the close distance between the farmers and UPTD-Bengkulu. These results are within the range of the research by Iswoyo and Widyaningrum (2006), who identified that the percentage of a good NRR was 79.53±18%. In addition to the quality of the sperm used, the skill of the inseminator and the number of acceptors also affect the NRR value. Some factors also affect the magnitude of the NRR value, such as the number of cows inseminated and the interval between the first inseminations.

Many factors can affect the results of artificial insemination. According to Yusuf *et al.* (2015), nutritional factors are one of the factors that greatly affect the reproductive performance of

Table 4. Non-return rate score of Bali cattle semen at Tanaq Mira Village

Farmer group	Number of acceptors (Head)	Return to estrous (Head)	NRR* (%)
Sapeng (Close)	5	1	80
Tunas Ridha Ilahi (Medium)	5	3	40
Sumber Rezeki (Far)	5	4	20
	Mean		46,67

*NRR observation was conducted on days 21-25 after insemination.

livestock. Most of the farmers in Tanaq Mira village feed their livestock only with forage without additional feed, either supplements or concentrates. Feeding is usually divided into three times a day. In the morning, farmers usually give field grass or elephant grass; then, in the afternoon, the feed given is still with the same feed, field grass or elephant grass added with corn waste, and in the last feeding in the late afternoon, farmers provide the same feed as the feed during the afternoon. Susilawati (2011) confirms that the re-emergence of estrus in NRR observations is also influenced by early embryonic death or inappropriate timing of IB implementation due to inaccurate information from farmer reports and caused by a lack of nutritional factors. Most of the farmer groups in Tanaq Mira Village, Wanasaba District, feed with fresh elephant grass and agricultural waste, especially corn, without providing additional feed to female cattle or pregnant mothers. According to (Suteky *et al.*, 2017), if the feed given is relatively the same, the feed factor is less influential in the NRR level, as well as the inseminator in the field, because there is only one inseminator on duty in the farmer group.

In addition to nutritional adequacy, environmental factors can also influence the occurrence of return to estrus after artificial insemination. Nuryadi and Wahjuningsih (2011) explain that the ability of female cows to become pregnant in the first insemination and not experience estrus again was strongly influenced by environmental variations. This is in line with the opinion of Rosita *et al.* (2014), who state that the success rate of AI is influenced by environmental factors such as temperature, climate, weather, and maintenance management, especially housing. With intensive maintenance, acceptor cows are rarely removed from the shed so that the cattle receive a low intensity of sunlight. It can trigger silent heat due to hormonal system disorders.

Conclusions

Based on the results of this research, it can be concluded that thawing time length based on distance has a very significant effect on the microscopic quality of postthaw frozen semen of Bali cattle, especially motility and viability, but has no significant effect on spermatozoa abnormalities. A thawing time of less than 10 minutes showed good motility and viability values and was suitable for artificial insemination, while a thawing time of more than 10 minutes could not be used for artificial insemination because of poor motility and viability values. The farther the distance between the AI station and the farmer group, the smaller the NRR value obtained.

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References

Adnyani, N. L. A., N. L. G. Sumardani, and N. P. Sarini. 2018. Pengaruh lama *thawing* pada uji kualitas *semen* beku Sapi Bali produksi UPT BIBD Baturiti sebelum didistribusikan. Peternakan Tropika 6: 626-636. https://ojs.unud.ac.id/index.php/tropika/article/view/42191

Afiati, F., Yulnawati, M. Riyadi, and R. I. Arifiantini. 2015. Abnormalitas spermatozoa domba dengan frekuensi penampungan berbeda. Prosiding Seminar 1: 930-934.

Andrefani, F., O. D. Putranti, and A. Hoda. 2019. Pengaruh lama *thawing* terhadap kualitas spermatozoa *semen* beku Sapi Bali (*Bos sondaicus*) di Dinas Pertanian Provinsi Maluku Utara. Jurnal Ilmu Peternakan (JANHUS) 3: 11-17. http://dx.doi.org/10.52434/janhus.v3i2.596

Ansari, M. S. Bushra, A. R. Syed, M. H. Andrabi, and A. Shamim. 2010. Effect of straw size and thawing time on quality of cryopreserved buffalo (*Bubalus bubalis*) *semen*. Reproductive Biology. 11: 49-54. https://doi.org/10.1016/s1642-431x(12)60063-1

Ariantie, O. S., Yusuf T. L., Sajuthi D. and Arifiantini R. I. 2013. Pengaruh krioprotektan gliserol dan dimethilformamida dalam pembekuan *semen* kambing PE menggunakan pengencer tris modifikasi. Jurnal Ilmu Ternak dan Veteriner 18: 239-250.

Arifiantini, R. I. 2012. Teknik Koleksi dan Evaluasi *Semen* pada Hewan. IPB Press, Bogor.

Arifiantini, R. I. and B. Purwantara. 2010. Motility and viability of Friesian Holstein spermatozoa in three different extender stored at 5^{o}C. Jurnal of the Indonesian Tropical Animal Agriculture 35: 222-226. https://doi.org/10.14710/jitaa.35.4.222-226

Badan Pusat Statistik Lombok Timur. 2018. Kecamatan Wanasaba Dalam Angka. CV. Maharani, Lombok Timur.

Fazrien, W. A., E. Herwijanti, and N. Isnaini. 2020. Pengaruh perbedaan individu terhadap kualitas *semen* segar dan beku pejantan unggul sapi Bali. Sains Peternakan 18: eISSN 2548-9321. https://doi.org/10.20961/sainspet.v18i1.37986

Fraser, L., J. Strzeżek, and W. Kordan. 2014. Post-thaw sperm characteristics following long-term storage of boar *semen* in liquid nitrogen. Anim. Reprod. Sci. 147: 119–127. https://doi.org/10.1016/j.anireprosci.2014.04.010

Gangawar, C., S. D. Kharche, S. Kumar and S. K. Jindal. 2016. Cryopreservation of goat

semen : status and prospects. Indian J. Small Rumin. 22: 1–10. http://dx.doi.org/10.5958/0973-9718.2016.00005.2

Gordon, I. 2017. Reproductive technologies in farm animals 2nd edn. University College Dublin, Ireland.

Hapsari, R. D., Y. Khalifah, N. Widyas, A. Pramono and S. Prastowo. 2018. Age effect on post freezing sperm viability of Bali cattle (*Bos javanicus*). IOP Conference Series: Earth and Environmental Science 142: 012007.

Irsan. 2015. Pengaruh lama *thawing* dan lama penyimpanan setelah *thawing* terhadap kualitas *semen* beku sapi. Skripsi. Sarjana Peternakan, Jurusan Produksi Ternak Fakultas Peternakan, Universitas Hasanudin, Makasar.

Iswoyo and P. Widiyaningrum. 2008. Performans reproduksi sapi Peranakan Simmental (PSM) hasil inseminasi buatan di Kabupaten Sukoharjo Jawa Tengah. Jurnal Ilmiah Ilmu-Ilmu Peternakan 11: 125-133. https://doi.org/10.22437/jiiip.v11i3.744

Jaelani, A., M. I. Zakir and A. Azis 2014. Pengaruh lama *thawing* dalam air es (3°c) terhadap persentase hidup dan motilitas spermatozoa Sapi Bali (*Bos sondaicus*). Jurnal Pena 26: http://dx.doi.org/10.31941/jurnalpena.v26i1.108

Jalius. 2011. Hubungan motilitas progresif dan keutuhan membran sperma dalam *semen* beku sapi Bali dengan keberhasilan inseminasi. Agribisnis Peternakan 1: 43-47.

Kementerian Pertanian. 2018. Konsumsi Produk Peternakan Per Kapita Per Tahun 2013-2017. Satistik Peternakan dan Kesehatan Hewan 2018. Hal 74. Nurdiman M dan Ramadhany AD. Direktorat Jenderal Peternakan dan Kesehatan Hewan. 978-979-628-035-3.

Khalil, W. A., M. A. El-Harairy, A. E. Zeidan, M. A. Hassan, and O. Mohey-Elsaeed. 2018. Evaluation of bull spermatozoa during and after cryopreservation: Structural and ultrastructural insights. Int. J. Vet. Sci. Med. 6: S49-S56. https://doi.org/10.1016/j.ijvsm.2017.11.001

Lopulalan F., T. Saili, and L. O. Baa. 2018. Kualitas dan fertilitas spermatozoa Sapi Bali hasil sexing dengan menggunakan metode swim-down. JITRO. 5: 24-33.

Mahfud, A., N. Isnaini, A. P. A. Yekti, Kuswati, and T. Susilawati. 2019. Kualitas spermatozoa post *thawing semen* beku sperma y hasil sexing pada Sapi Limousin. Jurnal Ternak Tropika 20: 1-7. https://doi.org/10.21776/ub.jtapro.2019.020.01.1

Nuryadi and S. Wahjuningsih. 2011. Penampilan reproduksi sapi Peranakan Ongole dan Peranakan Limousin di Kabupaten Malang. Jurnal Ternak Tropika 12: 76-81.

Prastika Z., S. Susilowati, B. Agustono, E. Safitri, F. Fikri and R. A. Prasitya. 2018. Motilitas dan viabilitas spermatozoa sapi Rambon di Desa Kemiren Banyuwangi. Jurnal Medik Veteriner 1: 38-42. http://dx.doi.org/10.20473/jmv.vol1.iss2.2018.38-42

Purwanti, M. and Harry. 2006. Upaya pemuliaan dan pelestarian sapi Bali di Provinsi Bali. Jurnal Penyuluhan Pertanian 1: 34-41. https://doi.org/10.51852/-.v1i1.196

Putranti, O. D. 2016. Pengaruh penambahan kafein pada sperma kauda epididimis sapi Bali pasca *thawing* terhadap fertilitas secara fertilisasi in vitro. Disertasi. Universitas Padjadjaran.

Rosita, E. A., T. Susilawati, and S. Wahyuningsih. 2014. Keberhasilan IB menggunakan *semen* beku hasil sexing dengan metode sedimentasi putih telur pada sapi PO cross. Jurnal Ilmu-Ilmu Peternakan 24: 72-76.

Saili, T., L. O. Naifu, L. O. Baa, S. Rahadi, A. Napirah, I. W. Sura and F. Lopulalan. 2017. Efektivitas sinkronisasi estrus dan fertilitas spermatozoa hasil sexing pada sapi Bali di Sulawesi Tenggara. Jurnal Veteriner 18: 1-7. https://doi.org/10.19087/jveteriner.2017.18.3.353

Salim, M. A., T. Susilawati, and S. Wahyuningsih. 2012. Pengaruh metode *thawing* terhadap kualitas *semen* beku Sapi Bali, sapi Madura dan sapi PO. J. Agripet 12: 14-19. https://doi.org/10.17969/agripet.v12i2.197

Samsudewa, D. and A. Suryawijaya. 2008. Pengaruh berbagai methode *thawing* terhadap kualitas *semen* beku sapi. Seminar Nasional Tekhnologi Peternakan dan Veteriner. Fakultas Peternakan Universitas Diponegoro, Semarang.

Soeparna and S. Nurcholidah. 2014. Ilmu Reproduksi Ternak. Institut Pertanian Bogor Press, Bogor.

Susilawati, T. 2011. Tingkat keberhasilan inseminasi buatan dengan kualitas dan deposisi *semen* yang berbeda pada sapi Peranakan Ongole. J. Ternak Tropika 12: 15-24.

Suteky, T. Sutriyono, Dwatmadji, and M. I. Sholihin. 2017. Kualitas *semen* produksi UPTD Bengkulu dan tingkat keberhasilan inseminasi pada sapi Bali dan Peranakan Simental di Bengkulu. Jurnal Sain Peternakan Indonesia 12: 221-229. https://doi.org/10.31186/jspi.id.12.2.221-229

Utami, T. and C. T. Tarsisius. 2014. Pengaruh suhu *thawing* pada kualitas spermatozoa sapi pejantan Friesian Holstein. JSV 32: 32-39.

Utomo, S. and E. Boquifai. 2010. Pengaruh temperatur dan lama *thawing* terhadap

kualitas spermatozoa sapi dalam penyimpanan straw beku. Sains Peternakan 1: 22-25. https://doi.org/10.20961/sainspet.v8i1.4740

Wahyuningsih, A., D. M. Saleh, and Sugiyanto. 2013. Pengaruh umur pejantan dan frekuensi penampungan terhadap volumedan motilitas *semen* segar sapi Simmental di Balai Inseminasi Buatan Lembang. Jurnal Ilmiah Peternakan 1: 947-953. http://jos.unsoed.ac.id/index.php/jip/article/view/679.

Witarsa. 2001. Evaluasi Semen. BIB Lembang, Bandung.

Yulnawati and M. A. Setiadi. 2005. Motilitas dan keutuhan membran plasma spermatozoa epididimis kucing selama penyimpanan pada suhu 4°C. Media Kedokteran Hewan 21: 100-104.

Yulnawati, Herdis, H. Maheswari, A. Boediono, and M. Rizal. 2009. Potensi reproduksi dan upaya pengembangbiakan kerbau Belang Tana Toraja. Seminar dan Lokakarya Nasional Kerbau. 152-158.

Yusuf, M. D. P. Rahardja and A. L. Toleng. 2015. Prospect of nutrition in-utero on improvement of reproductive performance in Bali cows kept under smallholder farms. J. Adv. Agric. Technol. 2: 151-155. https://doi.org/10.12720/joaat.2.2.151-155