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Influence of Method *Thawing* to Success Artificial Insemination of Cows

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ABSTRACT: Artificial insemination has been widely known among breeders as an effective reproductive technology by inserting sperm collected from male animals into the reproductive tract of female animals for the purpose of improving genetic quality. The success of AI is influenced by several factors, namely semen quality, inseminator expertise, livestock health, and other supporting factors. Good semen quality is one of the determining factors for the success of artificial insemination. One of the quality standards for frozen semen according to SNI is the level of motility *post thawing* minimum 40%. Motility level *post thawing* very dependent on technique *thawing* used. *Thawing* is the process of re-thawing frozen semen at physiological body temperature to reactivate sperm cells. *Thawing* can have an impact on reducing the quality of spermatozoa if not done properly. Temperature and time accuracy *thawing* will maintain sperm viability and motility, so that sperm quality is maintained and the success rate of artificial insemination will be high.

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INTRODUCTION

Artificial Insemination (AI) is well known and practiced by inseminators. Artificial insemination is considered effective in the field of reproduction. Apart from being able to improve genetic quality, breeders also do not have to increase the cost of having males to mate with their female livestock. The limited number of superior bulls in Indonesia is a problem in efforts to increase the population of superior cattle breeds to meet the demand for meat which is still insufficient. One way to increase the population with good and fast quality and quantity of livestock is by artificial mating or artificial insemination (AI). Artificial Insemination is an unnatural mating activity to bring together sperm cells and egg cells carried out on female livestock with human assistance. IB technique with input semen that has been melted and has been processed first from male cattle into the female genital tract (Dako *et al.*,2022).

AI is influenced by several factors, namely the accuracy of determining oestrus, inseminator expertise, semen quality, livestock health, and other supporting factors. In accordance with the literature, good semen quality is one of the determining factors for the success of artificial insemination (Putri *et al*., 2015). According to SNI standards, cement must have quality motility level*post thawing* minimum 40%, maximum percentage of abnormal spermatozoa 10%, minimum sperm concentration containing 25 million/*straw*, minimum individual movements 2. (SNI, 2017). Inseminators and breeders are the spearheads of implementation and are responsible for the success or failure of the artificial insemination program in the field (Widiati *et al*., 2008). Inaccuracy in technique can also cause IB failure so it can be one of the causes affecting the S/C value. S/C is an indicator of the reproductive performance of female livestock with values *service* (the insemination treatment is carried out) until the livestock becomes pregnant (Haryanto *et al.,* 2018). One of the main techniques that the inseminator needs to pay attention to is the handling method *thawing* before insemination into livestock. Method *thawing* Frozen semen is one of the most determining factors in artificial insemination. This is due to the use of methods *thawing* improper use will cause damage to spermatozoa, thereby reducing semen quality. *Thawing* it should be done at a physiological body temperature of around 37 – 38°C (Abdurrahman *et al.,* 2019). Sperm can experience *cold shock* due to drastic changes in temperature and in too short or too long a time. On the other hand, method *thawing* in some libraries it is very diverse, resulting in the use of methods *thawing* in the field is very diverse too. To produce good quality cement, the

Directorate General of Animal Husbandry has standardized the thawing method, namely using water at a temperature of 37°C for 30 seconds. However, the ease of implementation factor is a consideration for inseminators in implementation *thawing* (Primary *et al.*,2018).

LITERATURE REVIEW

Semen

Cattle semen in livestock breeding began at the beginning of the 20th century. Initially, the livestock breeding method was carried out by mating male cattle directly with female cattle. However, technological developments have made it possible to collect and process bovine semen, so that it can be stored and used more efficiently. This method is increasingly popular because it allows breeders to obtain offspring with the desired qualities and genetic traits (Ristiani *et al.*,2020). The quality of bull semen plays a very important role in mating, both natural and Artificial Insemination (AI).

There are many methods of semen collection that are often used, namely artificial vagina, massage of accessory genital organs, collection of semen from the vagina of female cows after mating (*post-coital*), electro ejaculator. Semen collection using an artificial vagina or *artificial vagina* Many people prefer it because it is simpler and has practical advantages and the results are more satisfying. Electro ejaculator is ejaculation obtained by electrical stimuli inserted into the rectum, the electrode will stimulate the central nervous system which stimulates the reproductive organs so that ejaculation occurs. This technique is of great value for males who cannot use an artificial vagina. The disadvantage of using this method is that it allows injury to the rectum, requires equipment such as electrodes, voltmeters, ammeters, etc. and the ability (*skill*) which is specifically for regulating the number of stimuli.

Semen collection by massaging the accessory genital organs involves palpation of the ampulla through the rectal wall, thus stimulating the release of semen. This method can be used on males that cannot climb or on collections that are incompletely equipped. However, this method requires special training. Usually the semen collected is contaminated with feces and urine, because the semen drips through the prepuce and prepuce hairs, so the fertility of the semen is low compared to using an artificial vagina. Therefore, the prepuce and the surrounding area must be washed with physiological NaCl solution or warm water. Then dry with a clean towel.

Semen quality can be influenced by race, age, feed, climate, animal health, type of feed consumed, breed and environment (Ristiani *et al.*,2020). Testing the quality of fresh semen can be carried out immediately after storage or before it is diluted by carrying out macroscopic and microscopic examinations. Macroscopic examination includes volume, color, concentration and pH. Microscopic examination includes mass motility, individual motility, live-dead percentage, concentration, and abnormalities.

Macroscopic examination shows that the normal volume of cow semen is between 1 – 15 ml, the color of normal cow semen is yellowish white, this is influenced by the riboflavin in the semen (Maksum *et al.*,2013). The color of semen is often confused with urine, but can be distinguished by smell. The degree of turbidity depends on the concentration of spermatozoa cells in the semen. The more cloudy there is, the greater the number of spermatozoa per millimeter. The degree of acidity (pH) of normal cow semen ranges from 6.4-7.8 (Ax *et al*., 2008; Garner and Hafez, 2008). Microscopic examination of spermatozoa motility is carried out after the semen is diluted or after *freezing* and *thawing*. Motility parameters include: the percentage of motile spermatozoa under normal conditions is 70 – 90% motile, the percentage of spermatozoa moving progressively, and the speed of spermatozoa (*velocity*) on a scale of 1-2 (fast) (Ax *et al*., 2008).

Post Thawing Motility (PTM)

Post Thawing Motility (PTM) is one of the aspects and procedures in evaluation *straw* cement. Frozen cement is obtained from the processing of fresh cement so that it lasts longer. PTM is an examination of sperm motility after it is thawed again. PTM is used to determine the level of sperm motility, whether it is still suitable for insemination (Azzahra *et al.*,2016). The PTM SNI standard set is a minimum of 40%. Method *thawing* Frozen cement is a very determining factor because of the method used *thawing* Improper use will cause damage to spermatozoa there by reducing semen quality. *Thawing* is the re-thawing of cement that has been frozen before carrying out (IB). Temperature and duration *thawing* has a great influence on the condition of spermatozoa, especially the integrity of spermatozoa in semen (Ma *et al.*,2019). Temperature combination *thawing* A good one is one that can prevent damage to spermatozoa, so that it still has a high ability to fertilize ovum. The best individual motility is the progressive movement of spermatozoa or active movement forward. Spermatozoa are susceptible to *cold shock* due to drastic changes in temperature. Signs *cold shock* or media that is not isotonic with cement causes circular movement and movement. Spermatozoa when they have stopped moving are considered dead (Feradis, 2010). Long time *thawing* will affect the percentage of spermatozoa viability. Duration *thawing* the longer it is, the percentage of spermatozoa viability will decrease, this is possibly caused by *cold shock* due to drastic changes in temperature (Salim *et al*., 2012). Sayoko *et al* (2007) stated that during *thawing* If temperature changes occur quickly it will reduce the pressure on the spermatozoa, thus helping sperm to pass through the critical phase quickly. *Cold shock* It can also cause spermatozoa abnormalities, with the characteristic tail and body parts circling the head (Salisbury and Van Demark, 1985). Abnormal spermatozoa will not be able to fertilize an ovum regardless of the abnormality category (Toelihere,

1993).

Artificial insemination

Artificial Insemination (AI) is a way to insert sperm into the female's reproductive tract using special methods and techniques. Artificial insemination is the first generation of livestock reproductive biotechnology in Indonesia and has been applied since 1956 (Kasehung *et al.*,2016). The aim of implementing AI is to increase the production and productivity (breeding) of livestock owned, by making maximum use of a superior male animal (Afiati *et al*., 2013).

Artificial insemination has the function of improving the genetic quality of livestock, preventing disease transmission, saving money on male maintenance, increasing the utilization of superior males, and shortening *calving interval* (Wodzicka-Tomaszewska *et al*., 1991 and Siahaan, 2012). The IB implementation procedure consists of observing lust, *handling* frozen cement, *thawing* frozen semen, as well as carrying out insemination (Samsudewa and Suryawijaya, 2008). AI can be said to be successful if the mother cow subjected to AI becomes pregnant until the fetus is born (Hastuti, 2008). The success of AI in livestock is determined by several factors, namely sperm quality, the condition of the female cow as an AI acceptor, accuracy of AI, and the skills of the implementer (inseminator). These factors are related to each other and if one of the values is low it will cause AI results to also be low, in the sense of abnormal production and reproductive efficiency (Toelihere, 1993).

One of the factors that influences the success of artificial insemination is the quality of the sperm to be injected. However, to make it last longer, sperm fluid is frozen and processed first. Frozen spermatozoa have the advantage of being able to be used for long periods of time, however this freezing affects the quality of spermatozoa, spermatozoa pass through various extreme temperatures which can reduce the quality of spermatozoa (Komariah *et al*., 2013). Freezing semen stops cell metabolism temporarily without killing the cells. The implementation of artificial insemination by inseminators and breeders is the spearhead of implementation and is responsible for the success or failure of the artificial insemination program in the field (Widiati *et al*., 2008). Things that need to be prepared before insemination are a female cow that is in heat, palpation gloves, *container straw*, *straw*, tweezers scissors, *plastic sheath*, *insemination gun.*

Container straw is a place to store *straw* which contains frozen cement, on the lid of the container, there is a hole used to take it *straw* with tweezers. iya*Container* contains nitrogen which functions to maintain the temperature so that the cement is not damaged, namely at a temperature of -196^{0}C.

Figure 1.*Container* (Acquainted *et al.*,2020)

Straw is the first place after the sperm is diluted. *Straw* in the form of a long straw with a cover at each end, namely *factory plug* and *laboratory plug. Factory plug* is the cover of the production plant *straw*, where as *laboratory plug* is the lid of the laboratory. On *straw*, given the stud code and ame of the production site

.**Figure 2. *Straw* (Acquainted *et al.*,2020)**

After*straw* put in*gun* and on the scissors, the next step is to install*plastic sheet*. Same as*straw*, *plastic sheath* shaped like a straw. However, it is longer and wider in diameter. At the end*plastic sheath*, there is a small cover that works to prevent*straw* out of*insemination gun* when the cement is fired.

Figure 3.*Plastic sheet* (Acquainted *et al.*,2020)

Gun or*insemination gun* is the main tool used to insert or shoot semen into the female reproductive tract.

Figure 4. *Gun* (Acquainted *et al.*,2020)

Cattle that are known to be in estrus are characterized by their vulva producing mucus, experiencing swelling, hyperemia, and looking restless within 12 – 18 hours and being prepared for AI. Preparation for AI of female cattle is done by tightening the cattle's reins and then tethering them to a stake. This is done to make it easier for the inseminator to carry out insemination. The Director General of Animal Husbandry (2012) stated that a cow in heat should be provided with a barrier by tying it up or clamping it so that the cow cannot move freely. After *insemination gun* ready, the inseminator will perform rectal palpation using the left hand which has been worn with a glove and has been wet with water and soap with the aim of makes palpation easier when the hand enters the rectum. The left hand is slowly inserted into the rectum with the palm in a conical position. If there is feces in the rectum, the rectum is cleaned first by removing the feces. After the rectum is clean, the left hand is inserted back into the rectum to carry out rectal palpation which aims to find the location of the cow's cervix so that semen can be deposited. Once the cervix is found, grasp the cervix and then insert it *insemination gun* from the vulva to the vagina to the cervix. Cement is then positioned at the base of the cervix.

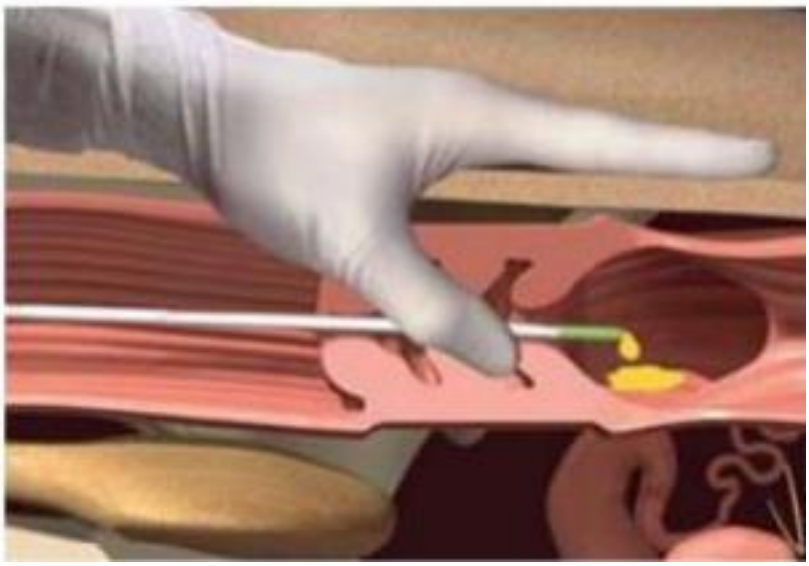

Figure 5. Cement deposition (Pasino *et al.*,2020)

In practice, artificial insemination has its own advantages and disadvantages. One of the drawbacks is that if you don't use the right procedures, it will result in a decrease in reproduction such as infection or abnormalities in the female genital tract. Apart from that, if you do not carry out strict selection of males, it will cause genetic abnormalities in the livestock. Several disease tests that are important to carry out before carrying out IB are brucellosis, campylobacteriosis, leptospirosis, trichomoniasis, tuberculosis and viral diarrhea (Susilawati, 2013). However, the advantage of AI will allow mating between livestock of different sizes without causing injury to males and females. Marriage is also not limited by time and place. Moreover, AI can maximize one superior male so that it can serve up to 5,000 – 10,000 females per year (Toelihere, 1993). Apart from that, AI allows cross breeding to be carried out to change production in a short time and allows the use of frozen semen so that the use of superior male semen is maximized even though the male has died (Warmadewi, 2014).

RESULTS AND DISCUSSION

Artificial insemination is a form of reproductive technology with a special method designed by inserting sperm collected from male animals into the reproductive tract of female animals for the purpose of improving genetic quality. This is supported by Putri *et al* (2015) stated that Artificial Insemination (AI) is a way to insert spermatozoa from male livestock into the female genital tract using special methods and tools. Fania *et al* (2020) stated that insemination is a series of processes planned and programmed because it concerns the genetic quality of livestock in the future. Afiati *et al* (2013) also stated that the aim of implementing AI is to increase the production and productivity (breeding) of livestock owned, by making maximum use of superior male animals (bulls). Good artificial insemination is a determining factor for pregnancy in animals. For this reason, it is necessary to pay attention to the factors that determine the success of artificial insemination, namely sperm quality, the condition of the female cow as an AI acceptor, accuracy of AI, animal health, and the skills of the implementing staff (inseminator). Good semen quality and methods are one of the determining factors for the success of artificial insemination. In accordance with the literature, good semen quality is one of the determining factors for the success of artificial insemination (Putri *et al.*,2015).

Cement can be used for a long time after going through the freezing process. However, when used for insemination, it needs to be diluted. *Thawing* is the process of re-thawing frozen semen to reactivate inactive cells. *Thawing* can have an impact on reducing the quality of spermatozoa. This is supported by Komariah *et al* (2013) who reported that frozen semen that is thawed during the freezing process will cause spermatozoa to undergo extreme temperatures which can reduce the quality of spermatozoa.

Apart from that, Apriyanti (2012) also stated that the process of freezing spermatozoa causes problems, namely the formation of ice crystals due to the process of releasing water intracellularly and *cold shock* or stress due to cold stress. The quality of spermatozoa will affect the results of IB. Semen quality decreases in terms of motility and viability as well as an increase in spermatozoa abnormalities. This is in line with research conducted by Janur *et al* (2019) stated that the method *thawing* Improper use can cause damage to spermatozoa, thereby reducing motility and viability as well as abnormalities in the shape of spermatozoa.

Method *thawing* in the field is very diverse, inseminators do *thawing* by using warm water, tap water, or holding it for a while. There are various techniques *thawing* which aims to liquefy the semen so that the sperm cells can be active again (Pratama *et al.*,2018). *Post Thawing Motility* This was done to determine the motility of spermatozoa after being frozen and diluted again. This examination is carried out after 24 – 48 hours post-process *free zing* and before distribution to consumers as a quality test. This is important to do to maintain quality *straw* into the hands of consumers. Supported by the literature of Susilawati (2013) that examination of spermatozoa motility after the phase *freezing* By making sperm temperature in physiological conditions, it is important to determine the level of sperm motility that will be distributed to the public. Method *thawing* Frozen semen is the most important procedure in artificial insemination. This is due to the use of methods *thawing* Improper use will cause spermatozoa damage, in accordance with the literature from Suryawijaya and Samsudewa (2008), damaged sperm will reduce semen quality. The percentage of spermatozoa movement is one of the benchmarks in assessing semen for artificial insemination, however, microscopic assessment of spermatozoa motility is subjective (Toelihere, 1993). The spermatozoa concentration requirement for standard artificial insemination is 2.5 million spermatozoa per *straw* with motility *post thawing* a minimum of 40% (SNI, 2017).

Temperature and age *thawing* has a great influence on the condition of spermatozoa, especially the integrity of spermatozoa in semen. Combination of temperature and time *thawing* Good ones can prevent damage to spermatozoa, so that sperm still have a high ability to fertilize ovum (Aprilina *et al.*,2019). Process *thawing* carried out at physiological body temperature to adapt sperm to actual conditions, namely at a temperature of 37-39°C within a few seconds. According to research conducted by Zelpina *et al* (2012) *thawing* Sperm tests were carried out at temperatures of 34°C, 37°C and 40°C for 15 seconds. It was found that at 37°C the results were better with a motility level of >40%. At temperatures of 34°C and 40°C sperm motility is <40%. this can happen because the sperm experiences *cold shock* due to drastic changes in temperature.

Research conducted by Aprilina *et al* (2019) who did *thawing* at 37°C for 10, 15, and 20 seconds. The research results show a long time *thawing* which is too short, namely 10 seconds, will result in low spermatozoa motility, this is due to the long time *thawing* which is too short causes the ice crystals to not melt completely, thus inhibiting the movement of spermatozoa cells actively. The same is true when it is old *thawing* Too long, namely 20 seconds, will cause low spermatozoa motility, because metabolic activity increases and occurs en masse, resulting in increased production of lactic acid. This is related to Darnel's opinion *et al* (1990) which causes spermatozoa to take too long to process *thawing* This will cause an increase in the production of lactic acid which is toxic to spermatozoa due to long-term metabolic activity of spermatozoa and there has been an increase in free radicals which produce lipid peroxidation as a factor causing damage. Motility for a long time *thawing* 15 seconds gives the best results because it allows the ice crystals to melt completely so that sperm activity can run optimally.

Semen motility will decrease over time until it is no longer suitable for insemination. The cause of decreased cement quality is lipid peroxidation. Lipids are important components in cell membranes (phospholipids, glycolipids and cholesterol). This component in the cell membrane contains polyunsaturated fatty acids which are very susceptible to oxidation which causes the formation of free radicals, especially hydroxyl radicals (OH-). These hydroxyl radicals can cause a chain reaction known as lipid peroxidation. The lipid peroxidation process occurs during the process *thawing*, until the process *thawing* Too long will cause more lipid peroxidation. This lipid peroxidation process will change the structure of spermatozoa, especially in the membrane and acrosome, they will lose motility, changes in metabolism and the release of intracellular components will increase spermatozoa damage resulting in increased spermatozoa death and decreased motility. This is in accordance with the opinion of Samsudewa and Suryawijaya (2008), that *thawing* Too long will cause a decrease in individual motility to a quality that can no longer be used for AI (<40%).

Temperature and time *thawing* influence on value *post thawing motality* (PTM). Temperatures that are too high and too low will affect the decrease in motility values. Time *thawing* Too short and too long will reduce the quality of the cement. This is supported by Susilawati (2011) who stated that the accuracy of temperature and time *thawing* will maintain cement quality. Good quality semen increases the chances of successful artificial insemination. So precision treatment is required *thawing* related to temperature and time which will affect the quality of semen and continue to the success of artificial insemination.

CONCLUSION

Based on the review of this paper, it can be concluded that 1) method *thawing* in the field varies greatly based on technique, temperature and time, 2) *Thawing* customized with physiological temperature and the right time will maintain sperm quality, 3) Good semen quality will increase the success of IB.

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