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Straw Quality and Success Rate of Artificial Insemination in Ongole Crossbred (PO) with Different Thawing Times

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ABSTRACT

Artificial insemination (AI) is the technique of semen injecting into a female cow's reproductive system without a natural mating. The aim of this study is to address the issue of how the thawing time affects the quality of the straw and the success AI. The study uses a bull batch 20904 L.92 code of 30 frozen semen from Ungaran (BIB) on 30 Ongole Crossbred cattle in Kepil region, Wonosobo, Central Java. Three treatments were applied, with microscopic evaluations to assess post-thawing spermatozoa quality, including motility and viability. Artificial insemination was performed on 30 cows Ongole Crossbred to see the service per conception and conception rate. Treatment T2 achieved maximum motility in 25 seconds, followed by T3 in 35 seconds, and T1 in 15 seconds. Long thawing times significantly affected frozen semen motility, with T2 significantly different from T1 and T3 respectively. Maximum frozen semen viability was achieved at treatment T2, T1, and T1. Long thawing times significantly affected frozen semen viability, with T2 significantly different from T1, T3, and T1. Long thawing times were not significant in post-thawing service per conception and conception rates from three treatments. Thawing for 25 seconds ensures high-quality sperm for successful artificial insemination

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INTRODUCTION

Artificial insemination (AI) in cattle refers to the process of introducing semen from a male bull into the reproductive tract of a female cow without natural mating. It is a widely used technique in cattle breeding and has several benefits, including genetic improvement, disease control, and increased reproductive efficiency [1]. AI allows cattle breeders to select superior bulls with desirable traits and genetics to sire offspring. This helps in improving the overall quality and productivity of the cattle population [2]. AI reduces the risk of transmitting sexually transmitted diseases and infections between animals during mating. It allows breeders to use semen from disease-free bulls, minimizing the spread of diseases within the herd [1]. By using AI, breeders can inseminate a larger number of cows with superior genetics from a single bull, maximizing the reproductive potential of the herd. This can lead to increased productivity and profitability.

Ongole Crossbred cattle are being developed and studied in Indonesia. The Indonesian government has implemented programs to develop Ongole Crossbred cattle farms in various regions, such as Gunungkidul Regency and Yogyakarta Special Region [3]. The Grati-Ongole Crossbred cattle (POGASI Agrinak) have been distributed in different agroecological zones in Indonesia, and their productive performance has been evaluated [4]. Climate change will also affect sustainable livestock farming, including PO cattle farming [5]. Genetic diversity of crossbred cattle, including Ongole Crossbred, has been studied in South Sulawesi, Indonesia, to establish conservation and breeding strategies [6]. The potential growth traits and semen quality rate of Ongole Crossbred bulls have also been studied in Tuban, Indonesia [7], [8]. Additionally, the polymorphism of the follicle-stimulating hormone beta subunit (FSH-P) gene has been studied as a molecular marker for reproductive status in Peranakan Ongole x Bali crossbred (POBA) cattle [9].

There are several studies related to AI in Ongole Crossbred cattle. Double-dose artificial insemination using sexed semen can result in a higher proportion of male calves [10], [11]. The success rate of double-dose artificial insemination at different times was evaluated in Ongole Crossbred cattle, and it was found that insemination at 2 and 8 hours after the estrus sign had better results than insemination at 8 and 16 hours [10]. The success of artificial insemination using unsexed sperm was compared to sexed sperm, and it was found that unsexed sperm showed better results [11]. Overall, these studies suggest that artificial insemination can be a valuable technique for improving cattle productivity in Indonesia and that factors such as timing and the type of sperm used can affect the success rate of the procedure.

Based on several studies that investigated the effect of thawing time on the success of artificial insemination in cattle, A study evaluated the success rate of double-dose artificial insemination at different times in Ongole Crossbred cattle. Two groups of cattle were inseminated at different time intervals after the estrus sign. The group inseminated at 2 and 8 hours after the estrus sign showed better results compared to the group inseminated at 8 and 16 hours. Parameters such as non-return rate (NRR), Conception Rate (CR), and Pregnancy Rate (PR) were measured [12]. The comparison of artificial insemination success between unsexed and sexed sperm was made using both types of frozen sperm. The results showed that unsexed sperm had better success rates in terms of non-return rate (NRR), Conception Rate (CR), and Pregnancy Rate (PR) compared to sexed sperm [13].

These studies suggest that thawing time can have an impact on the success of artificial insemination in cattle. Optimal thawing times may vary depending on the specific context and breed. It is important to consider factors such as temperature, duration, and the type of sperm used when determining the most suitable thawing conditions for artificial insemination in cattle. How the thawing time affects the quality of straw and the success of artificial insemination is the problem that is the goal of this research.

MATERIALS AND METHODS

The research material is a mini cemen frozen (straw) produced by Ungaran Artificial Insemination (BIB) with bull batch 20904 L.92 code of 30 pieces. Ongole Crossbred cattle (PO) at Kepil village, Wonosobo region, Central Java, which were selected through a purposive sampling method as a sample of the research, were 30 cows.

A sample was randomly divided into three treatments: T1 was a cow in insemination using straw with a long thawing time of 15 seconds; T2 was thawing for 30 seconds; and T3 was thawing for 35 seconds. The frozen semen (straw) was used for a microscopic quality evaluation as a form of post-thawing spermatozoa quality status such as motility and viability.

To calculate service per conception and calving rate, the following methods were used:

Service per conception (S/C) is calculated by dividing the number of services (inseminations) by the number of conceptions. For example, if a cow was inseminated three times and conceived once, the S/C would be 3. The calving rate is calculated by dividing the

number of calves born by the number of cows that were inseminated. For example, if 100 cows were inseminated and 80 calves were born, the calving rate would be 80% [14].

Services per Conception (S/C)

Services per Conception (S/C) is calculated from total pregnancy in the population. Based on [15] S/C is calculated:

$$\frac{Number\ of\ servicesinall\ pregnant}{total\ conceptions} \times 100\%$$

Conception Rate (CR)

Pregnancy Rate (PR) is the number of total pregnant cattle from the first and second insemination divided to total inseminated cattle in the herd. Based on [15] PR is calculated:

$$\frac{Total\ pregnancy\ cattel}{Total\ \ inseminated\ cattle} \times 100\%$$

RESULTS AND DISCUSSION

Kepil is one of the districts in Wonosobo, Central Java. It has an area of 9.386 ha and an altitude of 522 m. Information on cattle farming, breeds, or production specifically in Wonosobo is limited. A study on the optimum length of raising time and the relation with business income of Simmental-Ongole grade crossbred beef cattle fattening farm, aimed to analyze the optimum raising duration and its relationship with income in the business of Simmental-Ongole grade crossbred beef cattle. The study found that the Simmental Ongole crossbred had a higher carcass weight and carcass percentage, and feed cost per pound was more efficient when compared with Ongole-grade cattle [16].

Central Java is a prominent area for beef cattle production in Indonesia. The cattle population in Indonesia is currently about 16.6 million head, of which 43% are on Java Island [17]. In Central Java, crossbreeding with European breeds has been promoted to intensify beef production [18]. Crossbreeding at the farm level in Central Java showed that crossbreeding has not changed the farming systems or motivations for keeping cattle [18]. The study of thawing times on the quality of frozen semen and the success of artificial insemination is a study to add information to the PO cattle in Wonosobo.

Motility

Sperm motility is the ability of semen to move or swim actively after the process of freezing and storage. Good sperm motility is important in the reproductive process. Table 1 shows a good post-thawing rate, which is above 40%, which means the quality of the semen is worthy of insemination. Average maximum motility was achieved at treatment T2 (25 seconds), second T3 (35 seconds), and last T1 (15 seconds). Based on the results of the variance analysis showed significant for the long thawing times against the frozen semen motility. Further test results showed that T2 was different to T1 ($p < 0.05$), and T2 was significantly different to T3 ($p < 0.01$), while T1 and T3 are not different.

Table 1. Motility and vialability staw PO Bull batch 20904 L. 92 BIB Ungaran (%)

Treatments	Motility	Viability
T1	41[a]	57[a]
T2	49[b]	68[b]
T3	40[a]	52[c]

Different superscripts within a column denote significant differences.

Based on the research we've done, it turns out that sperm motility is best at 25 seconds of thawing (T2). A long-term thawing or freezing process can affect the quality of the semen used in artificial insemination. Long periods of thawing that are too short or too long can affect sperm motility. If the melting process is too fast, then the sperm may not have enough time to fully recover, so its motility may decrease. On the other hand, if the melting process is too long, the wrong temperature can damage the sperm and reduce its motility.

The time it takes to melt frozen semen ranges from 30 to 60 seconds at 37°C or 95°F [19], [20]. However, some sources recommend a shorter time, such as 45 seconds [20]. In addition to time, the temperature and method of melting also affect the quality of semen after in-thawing. Semen should be diluted in warm water at a temperature of 35–38 °C or 95–100 °F [21] [22]. In addition, semen should be kept safe from exposure to water, direct light, or too low temperatures. The semen that has been melted should be used within 5 to 15 minutes of melting to maximize the success of insemination [23]. Several factors can affect the motility of frozen-thawed semen, including genetics, breed, and cryopreservation. A study on the Brahman Cross Breeding Program found that genetics, breed, bull weight, bull age, and cryopreservation can cause a decrease in the quality of semen [24]. Another study on the cryopreservation of epididymal sperm found that total and slow progressive sperm motility, VCL, VAP, MAD, ALH, and BCF were significantly decreased after freezing and thawing [25]. Additionally, a study on breed differences in bull frozen-thawed semen found that BB bull spermatozoa have poorer progressive motility compared to Holstein Friesian [26].

Viability

Sperm viability refers to the ability of sperm to remain alive and active after the process of freezing and storage in the form of frozen semen. Sperm viability is an important factor in the success of artificial insemination or other reproductive techniques using frozen semen. Table 1 shows a good post-thawing rate of sperm viability from three treatments with scores of 57 (T1), 68 (T2), and 52 (T3). The viability of spermatozoa for the embossing of diluted or frozen semen must have a minimum of 60% to 75% of the living sperm [25]. Average maximum viability was achieved at treatment T2 (25 seconds), the second T1 (15 seconds), and the last T1 (35 seconds). Based on the results of the variance analysis, the long thawing times were significant for the frozen semen viability. Further test results showed that T2 was different from T1 ($p < 0.05$), significantly different from T3 ($p < 0.01$), and that T1 was different from T2 ($p < 0.05$) and was different from T3.

The ideal thawing time for frozen semen for artificial insemination in cows can vary depending on the thawing method used. Commercial breeding companies recommend different thawing methods for frozen semen in AI breeding, such as 35–38 °C for >30 seconds in the USA and 37.5–38.5 °C in Germany [27]. Thawing of frozen semen should be done at maximum speed to decrease the harmful effects of water recrystallization and rehydration, thus preventing damage to the sperm [19]. A study on bovine semen found that semen thawed at 28°C or 37°C can be used for AI until 90 minutes post-thaw [19]. Another thawing method described in the literature provides an alternative to the previously described thawing procedures containing a water bath: thawing frozen bull semen "in the cow." In this technique, the semen portion is taken from the liquid nitrogen container and inseminated immediately; thus, the semen is thawed right in the genital tract of the cow [27].

Semen quality serves as an indicator of fertility potential in Ongole Crossbred bulls, and there is a correlation between semen quality and fertility. Individual motility is an important factor in determining the success of artificial insemination, and bulls may vary in motility values [8]. A study evaluated the semen quality of Ongole Crossbred bulls and found that sperm viability in one treatment resulted in the highest value compared to other treatments [28]. Measured sperm motility in the liquid semen of Ongole Crossbred bulls and found that the diluent used can affect sperm motility. Overall, the viability of sperm in Ongole Crossbred bulls

is an important factor in semen quality and fertility potential. Different factors, such as individual variation and the diluent used, can influence sperm viability.

Services per Conception

Table 2 shows a post-thawing rate of service per conception (S/C) from three treatments with scores of 1.14 (T1), 1.12 (T2), and 1.14 (T3). Based on the results of the variance analysis, the long thawing times were not significant for the service per conception.

Table 2: Conception rate and pregnancy rate of Ongole Crossbred (PO)

Treatments	Services per Conception (S/C)	Conception Rate (CR)
T1	1.44	68
T2	1.42	70
T3	1.44	66

The amount of services per conception (S/C) in cattle is an important measure of reproductive performance. The standard number of services per conception (S/C) in cattle, is generally considered to range between 1.4 and 2.0. Rate of service per conception in the cows is an Ongole Crossbred in the clutches of 30 Kepil regions in good criteria. Achieving conception at the first service (FSC) after calving is crucial for improving reproductive performance and increasing profitability in dairy cows [27]. It is important to note that factors such as heat detection accuracy, semen handling, reproductive tract infection, nutritional status, fertility, and weather can influence the conception rate and subsequently the number of services per conception.

Service per conception (S/C) is a measure of reproductive efficiency in cattle that refers to the number of times a cow is inseminated before becoming pregnant [28], [29]. In Indonesia, there have been studies on the S/C of both beef and dairy cattle using artificial insemination. A study in Central Java found that the S/C of Indonesian Friesian Holstein cows was 1.71, meaning that one pregnancy required 1.71 times AI services [28]. Another study in Banyuwangi, East Java, measured S/C, conception rate, calving rate, and non-return rate in beef cattle [29]. A study in Central Kalimantan compared the reproductive efficiency of fertile female cattle using artificial insemination.

A study in Waru Sub-district, Pamekasan Regency, reported an average S/C of 1.55 ± 0.08 for Madura cattle in a special artificial insemination program [30]. The study examined beef cattle with ovarian hypofunction and repeat breeding. The cows with repeat breeding had an average S/C score of 4.15, which is higher than the standard S/C for Indonesian traditional breeders [29]. A study aimed to determine reproductive efficiency parameters in beef cattle. Although the specific average S/C was not mentioned, the study included S/C as one of the parameters analyzed [29]. On the success of double dose artificial insemination at different times in Ongole Crossbred cattle [12] The study found that the most effective thawing method for semen was 37°C for 10 seconds, and the S/C result indicated higher than expected pregnancy rates. Although the study did not specifically mention the influence of thawing time on S/C, it suggests that the thawing method can affect the success of artificial insemination. The thawing method can affect the success of artificial insemination, and different thawing methods may not have a significant effect on the conception rate.

Conception Rate

The conception rate in beef cattle refers to the percentage of cows or heifers that become pregnant during the breeding insemination. Table 2 shows a post-thawing rate of conception rate (CR) from three treatments with scores of 78 (T1), 80 (T2), and 76 (T3). The average

conception rate during the breeding season is around 94.9%, but it is not always 100% due to various factors such as management practices and technological advancements [20], [22].

First breeding season pregnancy rates in beef heifers can range from 64% to 95%. An ideal conception rate for beef cattle ranges from 65% to 70% [22]. The use of AI in combination with natural breeding (NB) has been shown to contribute to higher pregnancy rates in beef heifers [22]. It is important to note that the conception rate of beef heifers can still vary due to factors such as breed, individual animal characteristics, and environmental conditions. AI allows for the use of superior genetics and can increase the chances of successful conception.

The average conception rate of Madura cattle in the Special Artificial Insemination Program (SIWAB) in Waru Sub-district, Pamekasan Regency, was reported to be 66.0% ± 3.6% [30]. The genetic quality of beef cattle can affect their reproductive efficiency. Proper management practices can help improve the reproductive efficiency of beef cattle. A low-nutrition diet can negatively impact the physical condition of cows and reduce their pregnancy rate [31]. The conception rate of beef cattle can vary depending on the breed. A study on Madura cattle raised by small-scale farmers, did not report a specific conception rate [32].

An investigation of the time period within which frozen-thawed semen delivers a high conception rate in lactating dairy cows found that when AI was carried out 6–30 hours before ovulation, frozen-thawed semen delivered a conception rate of ≥ 60% [33]. Effect of different thawing methods for frozen bull semen and additional factors on the conception rate of dairy cows in artificial insemination found that thawing semen for >30 seconds can provide the best fertility results [34]. Time of insemination, and insemination technique in cattle found that numerous studies have been conducted to determine the thawing rate that gives the highest post-thaw percentage of viable sperm [20].

CONCLUSION

The conclusion of this study, the impact of thawing time on the quality of straw and the success of artificial insemination in Ongole Crossbred cattle. The study found that a thawing time of 25 seconds resulted in the highest sperm motility and viability rates. Thawing for too short or too long can affect sperm motility. If the thawing process is too fast, the sperm may not have enough time to fully recover, leading to decreased motility. On the other hand, if the thawing process is too long, the incorrect temperature can damage the sperm and reduce its motility. The thawing time of frozen semen in cattle is crucial for maintaining optimal sperm motility and viability. Thawing for the recommended time of around 25 seconds ensures the highest quality of sperm for successful artificial insemination.

Service per conception and conception rate are important measures of reproductive efficiency in cattle. Factors such as management practices, breed, individual animal characteristics, and environmental conditions can influence these measures. In this study, the thawing time did not affect the service per conception or the conception rate.

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